

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
14 June 2001 (14.06.2001)

PCT

(10) International Publication Number  
**WO 01/42304 A1**

(51) International Patent Classification<sup>7</sup>: **C07K 14/54**,  
14/545, 16/24, C12N 15/24, G01N 33/68, A61K 38/20

(21) International Application Number: PCT/US00/32940

(22) International Filing Date: 4 December 2000 (04.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/170,191	10 December 1999 (10.12.1999)	US
60/188,053	9 March 2000 (09.03.2000)	US
60/194,521	4 April 2000 (04.04.2000)	US
60/195,910	10 April 2000 (10.04.2000)	US
Not furnished	28 November 2000 (28.11.2000)	US

M. [NL/US]; 4027 Colonett Place, Newbury Park, CA 91320 (US). **GILES, Jennifer** [US/US]; 3031 Charlotte Street, Newbury Park, CA 91320 (US). **MU, Sharon, X.** [US/US]; 2954 Rikkard Drive, Thousand Oaks, CA 91362 (US). **XIA, Min** [CA/US]; 3946 Calle Buena Vista, Newbury Park, CA 91320 (US). **BASS, Michael, Brian** [US/US]; 1743 N. Marian Avenue, Thousand Oaks, CA 91360 (US). **CRAVEIRO, Roger** [US/US]; 384 East Wilbur Road, Apt. 203, Thousand Oaks, CA 91360 (US).

(74) Agent: **NOONAN, Kevin, E.**; McDonnell Boehnen Hulbert & Berghoff, 300 South Wacker Drive, Suite 3200, Chicago, IL 60606 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	60/170,191 (CON)
Filed on	10 December 1999 (10.12.1999)
US	60/188,053 (CON)
Filed on	9 March 2000 (09.03.2000)
US	60/194,521 (CON)
Filed on	4 April 2000 (04.04.2000)
US	60/195,910 (CON)
Filed on	10 April 2000 (10.04.2000)
US	Not furnished (CON)
Filed on	28 November 2000 (28.11.2000)

(71) Applicant (for all designated States except US): **AMGEN, INC.** [US/US]; One Amgen Center Drive, Thousand Oaks, CA 91320 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SARIS, Christiaan,**

(54) Title: INTERLEUKIN-1 RECEPTOR ANTAGONIST-RELATED MOLECULES AND USES THEREOF

(57) Abstract: The present invention provides novel Interleukin-1 Receptor Antagonist-Related (IL-1ra-R) polypeptides and nucleic acid molecules encoding the same. The invention also provides selective binding agents, vectors, host cells, and methods for producing IL-1ra-R polypeptides. The invention further provides pharmaceutical compositions and methods for the diagnosis, treatment, amelioration, and/or prevention of diseases, disorders, and conditions associated with IL-1ra-R polypeptides.

WO 01/42304 A1

## INTERLEUKIN-1 RECEPTOR ANTAGONIST-RELATED MOLECULES AND USES THEREOF

### Field of the Invention

5           The present invention relates to novel Interleukin-1 Receptor Antagonist-Related (IL-1ra-R) polypeptides and nucleic acid molecules encoding the same. The invention also relates to selective binding agents, vectors, host cells, and methods for producing IL-1ra-R polypeptides. The invention further relates to pharmaceutical compositions and methods for the diagnosis, treatment,  
10   amelioration, and/or prevention of diseases, disorders, and conditions associated with IL-1ra-R polypeptides.

### Background of the Invention

15           Technical advances in the identification, cloning, expression, and manipulation of nucleic acid molecules and the deciphering of the human genome have greatly accelerated the discovery of novel therapeutics. Rapid nucleic acid sequencing techniques can now generate sequence information at unprecedented rates and, coupled with computational analyses, allow the assembly of overlapping sequences into partial and entire genomes and the identification of  
20   polypeptide-encoding regions. A comparison of a predicted amino acid sequence against a database compilation of known amino acid sequences allows one to determine the extent of homology to previously identified sequences and/or structural landmarks. The cloning and expression of a polypeptide-encoding region of a nucleic acid molecule provides a polypeptide product for structural  
25   and functional analyses. The manipulation of nucleic acid molecules and encoded polypeptides may confer advantageous properties on a product for use as a therapeutic.

          In spite of the significant technical advances in genome research over the past decade, the potential for the development of novel therapeutics based on the  
30   human genome is still largely unrealized. Many genes encoding potentially beneficial polypeptide therapeutics or those encoding polypeptides, which may act

as "targets" for therapeutic molecules, have still not been identified.

Accordingly, it is an object of the invention to identify novel polypeptides, and nucleic acid molecules encoding the same, which have diagnostic or therapeutic benefit.

5

One of the most potent inflammatory cytokines yet discovered is interleukin-1 (IL-1). IL-1 is thought to be involved in many diseases and medical conditions. It is produced (though not exclusively) by cells of the macrophage/monocyte lineage, and may be produced in two forms: IL-1alpha  
10 (IL-1 $\alpha$ ) and IL-1beta (IL-1 $\beta$ ). Interleukin-1 receptor antagonist (IL-1ra) is a human protein that acts as a natural inhibitor of interleukin-1.

#### Summary of the Invention

The present invention relates to novel IL-1ra-R nucleic acid molecules and  
15 encoded polypeptides.

The invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- (a) the nucleotide sequence as set forth in any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35;
- 20 (b) the nucleotide sequence of the DNA insert in ATCC Deposit No. PTA-1423;
- (c) a nucleotide sequence encoding the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;
- (d) a nucleotide sequence which hybridizes under moderately or highly  
25 stringent conditions to the complement of any of (a) - (c); and
- (e) a nucleotide sequence complementary to any of (a) - (c).

The invention also provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

- 30 (a) a nucleotide sequence encoding a polypeptide which is at least about 70 percent identical to the polypeptide as set forth in any of SEQ ID NO: 2,

SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(b) a nucleotide sequence encoding an allelic variant or splice variant  
5 of the nucleotide sequence as set forth in any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, the nucleotide sequence of the DNA insert in ATCC Deposit No. PTA-1423, or (a);

(c) a region of the nucleotide sequence of any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, the DNA insert in ATCC Deposit  
10 No. PTA-1423, (a), or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide fragment has an activity of the encoded polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or is antigenic;

(d) a region of the nucleotide sequence of any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, the DNA insert in ATCC Deposit  
15 No. PTA-1423, or any of (a) - (c) comprising a fragment of at least about 16 nucleotides;

(e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (d); and

20 (f) a nucleotide sequence complementary to any of (a) - (d).

The invention further provides for an isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide as set forth in any of  
25 SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one conservative amino acid substitution, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(b) a nucleotide sequence encoding a polypeptide as set forth in any of  
30 SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one amino acid insertion, wherein the encoded polypeptide has an activity of the

polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(c) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least  
5 one amino acid deletion, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(d) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 which has a C-  
10 and/or N- terminal truncation, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(e) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least  
15 one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(f) a nucleotide sequence of any of (a) - (e) comprising a fragment of  
20 at least about 16 nucleotides;

(g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (f); and

(h) a nucleotide sequence complementary to any of (a) - (e).

25 The present invention provides for an isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36; and

(b) the amino acid sequence encoded by the DNA insert in ATCC  
30 Deposit No. PTA-1423.

The invention also provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

- (a) an amino acid sequence for an ortholog of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;
- 5 (b) an amino acid sequence which is at least about 70 percent identical to the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;
- 10 (c) a fragment of the amino acid sequence set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 comprising at least about 25 amino acid residues, wherein the fragment has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or is antigenic; and
- 15 (d) an amino acid sequence for an allelic variant or splice variant of the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, the amino acid sequence encoded by the DNA insert in ATCC Deposit No. PTA-1423, (a), or (b).

20 The invention further provides for an isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;
- 25 (b) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;
- 30 (c) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one amino acid

deletion, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(d) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 which has a C- and/or N- terminal truncation, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36; and

(e) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36.

The invention still further provides for an isolated polypeptide comprising the amino acid sequence as set forth in either SEQ ID NO: 1 or SEQ ID NO: 3 with at least one amino acid substitution selected from the group consisting of: arginine at position 2; alanine, lysine, or arginine at position 3; serine at position 7; lysine at position 8; alanine, cysteine, lysine, threonine, or serine at position 9; cysteine or phenylalanine at position 10; arginine or tryptophan at position 13; serine at position 15; arginine at position 18; serine or threonine at position 19; threonine at position 21; serine at position 23; arginine at position 34; tyrosine, serine, or arginine at position 37; lysine, arginine, threonine, or serine at position 38; threonine at position 41; serine, phenylalanine, or alanine at position 43; alanine at position 44; serine or lysine at position 48; alanine, threonine, or phenylalanine at position 52; serine at position 53; serine at position 54; alanine or tyrosine at position 58; lysine at position 65; phenylalanine at position 66; tyrosine at position 67; serine, tyrosine, or phenylalanine at position 69; lysine or serine at position 73; threonine or arginine at position 78; serine or alanine at position 90; alanine at position 91; serine at position 96; lysine or arginine at position 97; lysine or serine at position 98; alanine at position 100; tyrosine at position 102; arginine or alanine at position 104; lysine at position 105; threonine

at position 106; arginine at position 108; lysine, threonine, or trptophan at position 109; threonine or serine at position 110; serine at position 111; serine at position 114; serine at position 116; phenylalanine, cysteine, or tyrosine at position 117; tyrosine at position 121; serine or alanine at position 123; cysteine, serine, or threonine at position 126; serine at position 136; phenylalanine or arginine at position 138; threonine, arginine, or alanine at position 141; lysine or tyrosine at position 142; trptophan or threonine at position 143; alanine at position 145; threonine or serine at position 147; cysteine at position 151; and serine, cysteine, or phenylalanine at position 152; wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 6.

The invention still further provides for an isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 5 with at least one amino acid substitution selected from the group consisting of: arginine at position 21; alanine, lysine, or arginine at position 22; serine at position 26; lysine at position 27; alanine, cysteine, lysine, threonine, or serine at position 28; cysteine or phenylalanine at position 29; arginine or trptophan at position 32; serine at position 34; arginine at position 37; serine or threonine at position 38; threonine at position 40; serine at position 42; arginine at position 53; tyrosine, serine, or arginine at position 56; lysine, arginine, threonine, or serine at position 57; threonine at position 60; serine, phenylalanine, or alanine at position 62; alanine at position 63; serine or lysine at position 67; alanine, threonine, or phenylalanine at position 71; serine at position 72; serine at position 73; alanine or tyrosine at position 77; lysine at position 84; phenylalanine at position 85; tyrosine at position 86; serine, tyrosine, or phenylalanine at position 88; lysine or serine at position 92; threonine or arginine at position 97; serine or alanine at position 109; alanine at position 110; serine at position 115; lysine or arginine at position 116; lysine or serine at position 117; alanine at position 119; tyrosine at position 121; arginine or alanine at position 123; lysine at position 124; threonine at position 125; arginine at position 127; lysine, threonine, or trptophan at position 128; threonine or serine at position 129; serine at position 130; serine at position 133;



serine at position 135; phenylalanine, cysteine, or tyrosine at position 136; tyrosine at position 140; serine or alanine at position 142; cysteine, serine, or threonine at position 145; serine at position 155; phenylalanine or arginine at position 157; threonine, arginine, or alanine at position 160; lysine or tyrosine at position 161; trptophan or threonine at position 162; alanine at position 164; 5 threonine or serine at position 166; cysteine at position 170; and serine, cysteine, or phenylalanine at position 171; wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 6.

10 Also provided are fusion polypeptides comprising IL-1ra-R amino acid sequences.

The present invention also provides for an expression vector comprising the isolated nucleic acid molecules as set forth herein, recombinant host cells comprising the recombinant nucleic acid molecules as set forth herein, and a 15 method of producing an IL-1ra-R polypeptide comprising culturing the host cells and optionally isolating the polypeptide so produced.

A transgenic non-human animal comprising a nucleic acid molecule encoding an IL-1ra-R polypeptide is also encompassed by the invention. The IL-1ra-R nucleic acid molecules are introduced into the animal in a manner that 20 allows expression and increased levels of an IL-1ra-R polypeptide, which may include increased circulating levels. Alternatively, the IL-1ra-R nucleic acid molecules are introduced into the animal in a manner that prevents expression of endogenous IL-1ra-R polypeptide (*i.e.*, generates a transgenic animal possessing an IL-1ra-R polypeptide gene knockout). The transgenic non-human animal is 25 preferably a mammal, and more preferably a rodent, such as a rat or a mouse.

Also provided are derivatives of the IL-1ra-R polypeptides of the present invention.

Additionally provided are selective binding agents such as antibodies and peptides capable of specifically binding the IL-1ra-R polypeptides of the 30 invention. Such antibodies and peptides may be agonistic or antagonistic.

Pharmaceutical compositions comprising the nucleotides, polypeptides, or

selective binding agents of the invention and one or more pharmaceutically acceptable formulation agents are also encompassed by the invention. The pharmaceutical compositions are used to provide therapeutically effective amounts of the nucleotides or polypeptides of the present invention. The invention is also directed to methods of using the polypeptides, nucleic acid molecules, and selective binding agents.

The IL-1ra-R polypeptides and nucleic acid molecules of the present invention may be used to treat, prevent, ameliorate, and/or detect diseases and disorders, including those recited herein.

The present invention also provides a method of assaying test molecules to identify a test molecule that binds to an IL-1ra-R polypeptide. The method comprises contacting an IL-1ra-R polypeptide with a test molecule to determine the extent of binding of the test molecule to the polypeptide. The method further comprises determining whether such test molecules are agonists or antagonists of an IL-1ra-R polypeptide. The present invention further provides a method of testing the impact of molecules on the expression of IL-1ra-R polypeptide or on the activity of IL-1ra-R polypeptide.

Methods of regulating expression and modulating (*i.e.*, increasing or decreasing) levels of an IL-1ra-R polypeptide are also encompassed by the invention. One method comprises administering to an animal a nucleic acid molecule encoding an IL-1ra-R polypeptide. In another method, a nucleic acid molecule comprising elements that regulate or modulate the expression of an IL-1ra-R polypeptide may be administered. Examples of these methods include gene therapy, cell therapy, and anti-sense therapy as further described herein.

In another aspect of the present invention, the IL-1ra-R polypeptides may be used for identifying receptors thereof ("IL-1ra-R polypeptide receptors"). Various forms of "expression cloning" have been extensively used to clone receptors for protein ligands. *See, e.g.*, Simonsen and Lodish, 1994, *Trends Pharmacol. Sci.* 15:437-41 and Tartaglia *et al.*, 1995, *Cell* 83:1263-71. The isolation of an IL-1ra-R polypeptide receptor is useful for identifying or developing novel agonists and antagonists of the IL-1ra-R polypeptide signaling

pathway. Such agonists and antagonists include soluble IL-1ra-R polypeptide receptors, anti-IL-1ra-R polypeptide receptor-selective binding agents (such as antibodies and derivatives thereof), small molecules, and antisense oligonucleotides, any of which can be used for treating one or more disease or disorder, including those disclosed herein.

#### Brief Description of the Figures

Figures 1A-1B illustrate the nucleotide sequence of the human IL-1ra-R gene (SEQ ID NO: 1) and the deduced amino acid sequence of human IL-1ra-R polypeptide (SEQ ID NO: 2);

Figures 2A-2B illustrate the nucleotide sequence of a human IL-1ra-R gene variant (SEQ ID NO: 3) and the deduced amino acid sequence of the human IL-1ra-R polypeptide (SEQ ID NO: 4) encoded by this variant;

Figure 3 illustrates the nucleotide sequence of a human IL-1ra-R splice variant (SEQ ID NO: 5) and the deduced amino acid sequence of the human IL-1ra-R polypeptide (SEQ ID NO: 6) encoded by this splice variant;

Figures 4A-4B illustrate the amino acid sequence alignment of human IL-1 $\alpha$  (IL-1 $\alpha$ ; SEQ ID NO: 7), human IL-1 $\beta$  (IL-1 $\beta$ ; SEQ ID NO: 8), human IL-1 receptor antagonist (IL-1RA; SEQ ID NO: 9), human IL-1 $\delta$  (IL-1 $\delta$ ; SEQ ID NO: 10), human IL-1ra-R polypeptide (IL-1ra-R; SEQ ID NO: 2), human Tango-77 (Tango-77; SEQ ID NO: 11), human Zilla4 (Zilla4; SEQ ID NO: 12), human IL-1 $\zeta$  (IL-1 $\zeta$ ; SEQ ID NO: 13), human IL-1 receptor antagonist  $\beta$  (IL-1RA $\beta$ ; SEQ ID NO: 14), human SPOIL II (Spoil II; SEQ ID NO: 15), human IL-1 $\epsilon$  (IL-1 $\epsilon$ ; SEQ ID NO: 16), and human IL-1 $\eta$  (IL-1 $\eta$ ; SEQ ID NO: 17);

Figure 5 schematically illustrates the phylogenetic relationship of the IL-1ra gene family.

Figure 6 schematically illustrates the relationship between human IL-1ra-R polypeptide (Mature CS329), the sequence variant of human IL-1ra-R polypeptide (Mature CS329 Variant protein), and the splice variant of human IL-1ra-R polypeptide (Omega 329 protein);

Figure 7 illustrates the nucleotide sequence of the murine IL-1ra-R gene (SEQ ID NO: 35) and the deduced amino acid sequence of murine IL-1ra-R polypeptide (SEQ ID NO: 36);

Figure 8 illustrates the amino acid sequence alignment of human IL-1ra-R polypeptide (huIL-1ra-R; SEQ ID NO: 2) and murine IL-1ra-R polypeptide (muIL-1ra-R; SEQ ID NO: 36);

Figures 9A-9I illustrate the genomic nucleotide sequence for the murine IL-1ra-R gene (SEQ ID NO: 37). The locations of the coding portions of exons 1-4 are indicated (underline);

Figures 10A-10C illustrate the expression of murine (Figure 10A) and human (Figures 10B-10C) IL-1ra-R mRNA as detected by Northern blot analysis;

Figures 11A-11B illustrate the results of Western blot analysis using an anti-IL-1ra-R antibody;

Figure 12 illustrates the results of FACS analysis of spleen and bone marrow cells recovered from lethally irradiated recipient mice transplanted with murine bone marrow cells transduced with a retroviral vector containing the IL-1ra-R gene. The percentage of cells within the lymphoid compartment is shown for cells subjected to standard FACS analysis using the indicated cell surface markers;

Figures 13A-13B illustrate the results of colony assays performed on spleen and

bone marrow cells recovered from lethally irradiated recipient mice transplanted with murine bone marrow cells transduced with a retroviral vector containing the IL-1ra-R gene. Bone marrow and spleen cells from transduced and control mice were cultured under standard colony assay conditions and colonies were counted  
5 on day 14;

Figure 14 illustrates the production of  $\gamma$ -interferon in response to IL-12 treatment in spleen cells recovered from lethally irradiated recipient mice transplanted with murine bone marrow cells transduced with a retroviral vector containing the IL-  
10 1ra-R gene. A sample of conditioned media was removed from cultures ( $2 \times 10^6$  cells/well/ml) grown in the presence of IL-12 (1 ng/ml) for 48 hours and IFN- $\gamma$  was quantitated by ELISA;

Figure 15 illustrates the production of  $\gamma$ -interferon in response to IL-12 and IL-18  
15 treatment in spleen cells recovered from lethally irradiated recipient mice transplanted with murine bone marrow cells transduced with a retroviral vector containing the IL-1ra-R gene. A sample of conditioned media was removed from cultures ( $2 \times 10^6$  cells/well/ml) grown in the presence of IL-12 (1 ng/ml) and IL-18 (10 ng/ml) for 48 hours and IFN- $\gamma$  was quantitated by ELISA.

20

#### Detailed Description of the Invention

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All references cited in this application are expressly incorporated by reference herein.

25

#### Definitions

The terms "IL-1ra-R gene" or "IL-1ra-R nucleic acid molecule" or "IL-1ra-R polynucleotide" refer to a nucleic acid molecule comprising or consisting of a nucleotide sequence as set forth in any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ  
30 ID NO: 5, or SEQ ID NO: 35, a nucleotide sequence encoding the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO:

36, a nucleotide sequence of the DNA insert in ATCC Deposit No. PTA-1423, and nucleic acid molecules as defined herein.

The term "IL-1ra-R polypeptide allelic variant" refers to one of several possible naturally occurring alternate forms of a gene occupying a given locus on a chromosome of an organism or a population of organisms.

The term "IL-1ra-R polypeptide splice variant" refers to a nucleic acid molecule, usually RNA, which is generated by alternative processing of intron sequences in an RNA transcript of IL-1ra-R polypeptide amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36.

The term "isolated nucleic acid molecule" refers to a nucleic acid molecule of the invention that (1) has been separated from at least about 50 percent of proteins, lipids, carbohydrates, or other materials with which it is naturally found when total nucleic acid is isolated from the source cells, (2) is not linked to all or a portion of a polynucleotide to which the "isolated nucleic acid molecule" is linked in nature, (3) is operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature as part of a larger polynucleotide sequence. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from any other contaminating nucleic acid molecule(s) or other contaminants that are found in its natural environment that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use.

The term "nucleic acid sequence" or "nucleic acid molecule" refers to a DNA or RNA sequence. The term encompasses molecules formed from any of the known base analogs of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinyl-cytosine, pseudoisocytosine, 5-(carboxyhydroxymethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-iso-pentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-

methycytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyamino-methyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonyl-methyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

The term "vector" is used to refer to any molecule (e.g., nucleic acid, plasmid, or virus) used to transfer coding information to a host cell.

The term "expression vector" refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control the expression of inserted heterologous nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and RNA splicing, if introns are present.

The term "operably linked" is used herein to refer to an arrangement of flanking sequences wherein the flanking sequences so described are configured or assembled so as to perform their usual function. Thus, a flanking sequence operably linked to a coding sequence may be capable of effecting the replication, transcription and/or translation of the coding sequence. For example, a coding sequence is operably linked to a promoter when the promoter is capable of directing transcription of that coding sequence. A flanking sequence need not be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

The term "host cell" is used to refer to a cell which has been transformed, or is capable of being transformed with a nucleic acid sequence and then of expressing a selected gene of interest. The term includes the progeny of the parent cell, whether or not the progeny is identical in morphology or in genetic make-up to the original parent, so long as the selected gene is present.

The term "IL-1ra-R polypeptide" refers to a polypeptide comprising the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 and related polypeptides. Related polypeptides include IL-1ra-R polypeptide fragments, IL-1ra-R polypeptide orthologs, IL-1ra-R polypeptide variants, and IL-1ra-R polypeptide derivatives, which possess at least one activity of the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. IL-1ra-R polypeptides may be mature polypeptides, as defined herein, and may or may not have an amino-terminal methionine residue, depending on the method by which they are prepared.

10 The term "IL-1ra-R polypeptide fragment" refers to a polypeptide that comprises a truncation at the amino-terminus (with or without a leader sequence) and/or a truncation at the carboxyl-terminus of the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. The term "IL-1ra-R polypeptide fragment" also refers to amino-terminal and/or carboxyl-  
15 terminal truncations of IL-1ra-R polypeptide orthologs, IL-1ra-R polypeptide derivatives, or IL-1ra-R polypeptide variants, or to amino-terminal and/or carboxyl-terminal truncations of the polypeptides encoded by IL-1ra-R polypeptide allelic variants or IL-1ra-R polypeptide splice variants. IL-1ra-R polypeptide fragments may result from alternative RNA splicing or from *in vivo*  
20 protease activity. Membrane-bound forms of an IL-1ra-R polypeptide are also contemplated by the present invention. In preferred embodiments, truncations and/or deletions comprise about 10 amino acids, or about 20 amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or more than about 100 amino acids. The polypeptide fragments so produced will comprise  
25 about 25 contiguous amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or about 125 amino acids. Such IL-1ra-R polypeptide fragments may optionally comprise an amino-terminal methionine residue. It will be appreciated that such fragments can be used, for example, to generate antibodies to IL-1ra-R polypeptides.

30 The term "IL-1ra-R polypeptide ortholog" refers to a polypeptide from another species that corresponds to IL-1ra-R polypeptide amino acid sequence as



set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. For example, mouse and human IL-1ra-R polypeptides are considered orthologs of each other.

The term "IL-1ra-R polypeptide variants" refers to IL-1ra-R polypeptides comprising amino acid sequences having one or more amino acid sequence substitutions, deletions (such as internal deletions and/or IL-1ra-R polypeptide fragments), and/or additions (such as internal additions and/or IL-1ra-R fusion polypeptides) as compared to the IL-1ra-R polypeptide amino acid sequence set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 (with or without a leader sequence). Variants may be naturally occurring (e.g., IL-1ra-R polypeptide allelic variants, IL-1ra-R polypeptide orthologs, and IL-1ra-R polypeptide splice variants) or artificially constructed. Such IL-1ra-R polypeptide variants may be prepared from the corresponding nucleic acid molecules having a DNA sequence that varies accordingly from the DNA sequence as set forth in any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35. In preferred embodiments, the variants have from 1 to 3, or from 1 to 5, or from 1 to 10, or from 1 to 15, or from 1 to 20, or from 1 to 25, or from 1 to 50, or from 1 to 75, or from 1 to 100, or more than 100 amino acid substitutions, insertions, additions and/or deletions, wherein the substitutions may be conservative, or non-conservative, or any combination thereof.

The term "IL-1ra-R polypeptide derivatives" refers to the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, IL-1ra-R polypeptide fragments, IL-1ra-R polypeptide orthologs, or IL-1ra-R polypeptide variants, as defined herein, that have been chemically modified. The term "IL-1ra-R polypeptide derivatives" also refers to the polypeptides encoded by IL-1ra-R polypeptide allelic variants or IL-1ra-R polypeptide splice variants, as defined herein, that have been chemically modified.

The term "mature IL-1ra-R polypeptide" refers to an IL-1ra-R polypeptide lacking a leader sequence. A mature IL-1ra-R polypeptide may also include other modifications such as proteolytic processing of the amino-terminus (with or without a leader sequence) and/or the carboxyl-terminus, cleavage of a smaller

polypeptide from a larger precursor, N-linked and/or O-linked glycosylation, and the like.

The term "IL-1ra-R fusion polypeptide" refers to a fusion of one or more amino acids (such as a heterologous protein or peptide) at the amino- or carboxyl-terminus of the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, IL-1ra-R polypeptide fragments, IL-1ra-R polypeptide orthologs, IL-1ra-R polypeptide variants, or IL-1ra-R derivatives, as defined herein. The term "IL-1ra-R fusion polypeptide" also refers to a fusion of one or more amino acids at the amino- or carboxyl-terminus of the polypeptide encoded by IL-1ra-R polypeptide allelic variants or IL-1ra-R polypeptide splice variants, as defined herein.

The term "biologically active IL-1ra-R polypeptides" refers to IL-1ra-R polypeptides having at least one activity characteristic of the polypeptide comprising the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. In addition, an IL-1ra-R polypeptide may be active as an immunogen; that is, the IL-1ra-R polypeptide contains at least one epitope to which antibodies may be raised.

The term "isolated polypeptide" refers to a polypeptide of the present invention that (1) has been separated from at least about 50 percent of polynucleotides, lipids, carbohydrates, or other materials with which it is naturally found when isolated from the source cell, (2) is not linked (by covalent or noncovalent interaction) to all or a portion of a polypeptide to which the "isolated polypeptide" is linked in nature, (3) is operably linked (by covalent or noncovalent interaction) to a polypeptide with which it is not linked in nature, or (4) does not occur in nature. Preferably, the isolated polypeptide is substantially free from any other contaminating polypeptides or other contaminants that are found in its natural environment that would interfere with its therapeutic, diagnostic, prophylactic or research use.

The term "identity," as known in the art, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by comparing the sequences. In the art, "identity" also

means the degree of sequence relatedness between nucleic acid molecules or polypeptides, as the case may be, as determined by the match between strings of two or more nucleotide or two or more amino acid sequences. "Identity" measures the percent of identical matches between the smaller of two or more  
5 sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (*i.e.*, "algorithms").

The term "similarity" is a related concept, but in contrast to "identity," "similarity" refers to a measure of relatedness which includes both identical matches and conservative substitution matches. If two polypeptide sequences  
10 have, for example, 10/20 identical amino acids, and the remainder are all non-conservative substitutions, then the percent identity and similarity would both be 50%. If in the same example, there are five more positions where there are conservative substitutions, then the percent identity remains 50%, but the percent similarity would be 75% (15/20). Therefore, in cases where there are conservative  
15 substitutions, the percent similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

The term "naturally occurring" or "native" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by  
20 man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The terms "effective amount" and "therapeutically effective amount" each refer to the amount of an IL-1ra-R polypeptide or IL-1ra-R nucleic acid molecule  
25 used to support an observable level of one or more biological activities of the IL-1ra-R polypeptides as set forth herein.

The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of the IL-1ra-R polypeptide,  
30 IL-1ra-R nucleic acid molecule, or IL-1ra-R selective binding agent as a pharmaceutical composition.

The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antibody, and additionally capable of being used in an animal to produce antibodies capable of binding to an epitope of that antigen. An antigen may have one or more epitopes.

5       The term "selective binding agent" refers to a molecule or molecules having specificity for an IL-1ra-R polypeptide. As used herein, the terms, "specific" and "specificity" refer to the ability of the selective binding agents to bind to human IL-1ra-R polypeptides and not to bind to human non-IL-1ra-R polypeptides. It will be appreciated, however, that the selective binding agents  
10       may also bind orthologs of the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, that is, interspecies versions thereof, such as mouse and rat IL-1ra-R polypeptides.

      The term "transduction" is used to refer to the transfer of genes from one bacterium to another, usually by a phage. "Transduction" also refers to the  
15       acquisition and transfer of eukaryotic cellular sequences by retroviruses.

      The term "transfection" is used to refer to the uptake of foreign or exogenous DNA by a cell, and a cell has been "transfected" when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art and are disclosed herein. See, e.g., Graham  
20       *et al.*, 1973, *Virology* 52:456; Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratories, 1989); Davis *et al.*, *Basic Methods in Molecular Biology* (Elsevier, 1986); and Chu *et al.*, 1981, *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

25       The term "transformation" as used herein refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain a new DNA. For example, a cell is transformed where it is genetically modified from its native state. Following transfection or transduction, the transforming DNA may recombine with that of the cell by physically integrating  
30       into a chromosome of the cell, may be maintained transiently as an episomal element without being replicated, or may replicate independently as a plasmid. A

cell is considered to have been stably transformed when the DNA is replicated with the division of the cell.

Relatedness of Nucleic Acid Molecules and/or Polypeptides

5           It is understood that related nucleic acid molecules include allelic or splice variants of the nucleic acid molecule of any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, and include sequences which are complementary to any of the above nucleotide sequences. Related nucleic acid molecules also include a nucleotide sequence encoding a polypeptide comprising  
10 or consisting essentially of a substitution, modification, addition and/or deletion of one or more amino acid residues compared to the polypeptide in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. Such related IL-1ra-R polypeptides may comprise, for example, an addition and/or a deletion of one or more N-linked or O-linked glycosylation sites or an addition and/or a deletion of  
15 one or more cysteine residues.

          Related nucleic acid molecules also include fragments of IL-1ra-R nucleic acid molecules which encode a polypeptide of at least about 25 contiguous amino acids, or about 50 amino acids, or about 75 amino acids, or about 100 amino acids, or about 125 amino acids, or more than 125 amino acid residues of the IL-  
20 1ra-R polypeptide of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36.

          In addition, related IL-1ra-R nucleic acid molecules also include those molecules which comprise nucleotide sequences which hybridize under moderately or highly stringent conditions as defined herein with the fully  
25 complementary sequence of the IL-1ra-R nucleic acid molecule of any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, or of a molecule encoding a polypeptide, which polypeptide comprises the amino acid sequence as shown in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or of a nucleic acid fragment as defined herein, or of a nucleic acid fragment  
30 encoding a polypeptide as defined herein. Hybridization probes may be prepared using the IL-1ra-R sequences provided herein to screen cDNA, genomic or

synthetic DNA libraries for related sequences. Regions of the DNA and/or amino acid sequence of IL-1ra-R polypeptide that exhibit significant identity to known sequences are readily determined using sequence alignment algorithms as described herein and those regions may be used to design probes for screening.

5           The term "highly stringent conditions" refers to those conditions that are designed to permit hybridization of DNA strands whose sequences are highly complementary, and to exclude hybridization of significantly mismatched DNAs. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of  
10 "highly stringent conditions" for hybridization and washing are 0.015 M sodium chloride, 0.0015 M sodium citrate at 65-68°C or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 50% formamide at 42°C. See Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory, 1989); Anderson *et al.*, *Nucleic Acid Hybridisation: A Practical  
15 Approach* Ch. 4 (IRL Press Limited).

More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used — however, the rate of hybridization will be affected. Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific  
20 and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate, NaDodSO<sub>4</sub>, (SDS), ficoll, Denhardt's solution, sonicated salmon sperm DNA (or another non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these  
25 additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4; however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson *et al.*, *Nucleic Acid Hybridisation: A Practical Approach* Ch. 4 (IRL Press Limited).

30           Factors affecting the stability of DNA duplex include base composition, length, and degree of base pair mismatch. Hybridization conditions can be

adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids. The melting temperature of a perfectly matched DNA duplex can be estimated by the following equation:

5 
$$T_m(^{\circ}\text{C}) = 81.5 + 16.6(\log[\text{Na}^+]) + 0.41(\% \text{G+C}) - 600/N - 0.72(\% \text{formamide})$$

where N is the length of the duplex formed, [Na<sup>+</sup>] is the molar concentration of the sodium ion in the hybridization or washing solution, %G+C is the percentage of (guanine+cytosine) bases in the hybrid. For imperfectly matched hybrids, the melting temperature is reduced by approximately 1°C for each 1% mismatch.

10 The term "moderately stringent conditions" refers to conditions under which a DNA duplex with a greater degree of base pair mismatching than could occur under "highly stringent conditions" is able to form. Examples of typical "moderately stringent conditions" are 0.015 M sodium chloride, 0.0015 M sodium citrate at 50-65°C or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 20%  
15 formamide at 37-50°C. By way of example, "moderately stringent conditions" of 50°C in 0.015 M sodium ion will allow about a 21% mismatch.

It will be appreciated by those skilled in the art that there is no absolute distinction between "highly stringent conditions" and "moderately stringent conditions." For example, at 0.015 M sodium ion (no formamide), the melting  
20 temperature of perfectly matched long DNA is about 71°C. With a wash at 65°C (at the same ionic strength), this would allow for approximately a 6% mismatch. To capture more distantly related sequences, one skilled in the art can simply lower the temperature or raise the ionic strength.

A good estimate of the melting temperature in 1M NaCl\* for  
25 oligonucleotide probes up to about 20nt is given by:

$$T_m = 2^{\circ}\text{C per A-T base pair} + 4^{\circ}\text{C per G-C base pair}$$

\*The sodium ion concentration in 6X salt sodium citrate (SSC) is 1M. See Suggs *et al.*, *Developmental Biology Using Purified Genes* 683 (Brown and Fox, eds., 1981).

30 High stringency washing conditions for oligonucleotides are usually at a temperature of 0-5°C below the T<sub>m</sub> of the oligonucleotide in 6X SSC, 0.1% SDS.

In another embodiment, related nucleic acid molecules comprise or consist of a nucleotide sequence that is at least about 70 percent identical to the nucleotide sequence as shown in any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, or comprise or consist essentially of a nucleotide sequence  
5 encoding a polypeptide that is at least about 70 percent identical to the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. In preferred embodiments, the nucleotide sequences are about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the nucleotide sequence as shown in any  
10 of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, or the nucleotide sequences encode a polypeptide that is about 75 percent, or about 80 percent, or about 85 percent, or about 90 percent, or about 95, 96, 97, 98, or 99 percent identical to the polypeptide sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. Related nucleic acid  
15 molecules encode polypeptides possessing at least one activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36.

Differences in the nucleic acid sequence may result in conservative and/or non-conservative modifications of the amino acid sequence relative to the amino  
20 acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36.

Conservative modifications to the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 (and the corresponding modifications to the encoding nucleotides) will produce a polypeptide having  
25 functional and chemical characteristics similar to those of IL-1ra-R polypeptides. In contrast, substantial modifications in the functional and/or chemical characteristics of IL-1ra-R polypeptides may be accomplished by selecting substitutions in the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 that differ significantly in their effect on  
30 maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or



hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis."

Conservative amino acid substitutions also encompass non-naturally occurring amino acid residues that are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics, and other reversed or inverted forms of amino acid moieties.

Naturally occurring residues may be divided into classes based on common side chain properties:

- 1) hydrophobic: norleucine, Met, Ala, Val, Leu, Ile;
- 2) neutral hydrophilic: Cys, Ser, Thr;
- 3) acidic: Asp, Glu;
- 4) basic: Asn, Gln, His, Lys, Arg;
- 5) residues that influence chain orientation: Gly, Pro; and
- 6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human IL-1ra-R polypeptide that are homologous with non-human IL-1ra-R polypeptides, or into the non-homologous regions of the molecule.

In making such changes, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. The hydropathic indices are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-

3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte *et al.*, 1982, *J. Mol. Biol.* 157:105-31). It is known that certain amino acids may  
5 be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within  $\pm 2$  is preferred, those which are within  $\pm 1$  are particularly preferred, and those within  $\pm 0.5$  are even more particularly preferred.

10 It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functionally equivalent protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its  
15 adjacent amino acids, correlates with its immunogenicity and antigenicity, *i.e.*, with a biological property of the protein.

The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate ( $+3.0 \pm 1$ ); glutamate ( $+3.0 \pm 1$ ); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-  
20 0.4); proline ( $-0.5 \pm 1$ ); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within  $\pm 2$  is preferred, those which are within  $\pm 1$  are particularly preferred,  
25 and those within  $\pm 0.5$  are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such  
30 substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the IL-1ra-R polypeptide, or to increase or decrease

the affinity of the IL-1ra-R polypeptides described herein. Exemplary amino acid substitutions are set forth in Table I.

Table I

5

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe

Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu
-----	--	-----

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 using well-known techniques. For identifying suitable areas of the molecule that may be changed without destroying biological activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of an IL-1ra-R polypeptide to such similar polypeptides. With such a comparison, one can identify residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of the IL-1ra-R molecule that are not conserved relative to such similar polypeptides would be less likely to adversely affect the biological activity and/or structure of an IL-1ra-R polypeptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in an IL-1ra-R polypeptide that correspond to amino acid residues that are important for activity or structure in similar polypeptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of IL-1ra-R polypeptides.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view

of such information, one skilled in the art may predict the alignment of amino acid residues of IL-1ra-R polypeptide with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each amino acid residue. The variants could be screened using activity assays known to those with skill in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moulton, 1996, *Curr. Opin. Biotechnol.* 7:422-27; Chou *et al.*, 1974, *Biochemistry* 13:222-45; Chou *et al.*, 1974, *Biochemistry* 113:211-22; Chou *et al.*, 1978, *Adv. Enzymol. Relat. Areas Mol. Biol.* 47:45-48; Chou *et al.*, 1978, *Ann. Rev. Biochem.* 47:251-276; and Chou *et al.*, 1979, *Biophys. J.* 26:367-84. Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40%, often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within the structure of a polypeptide or protein. See Holm *et al.*, 1999, *Nucleic Acids Res.* 27:244-47. It has been suggested that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate (Brenner *et al.*, 1997, *Curr. Opin. Struct. Biol.* 7:369-76).

Additional methods of predicting secondary structure include "threading" (Jones, 1997, *Curr. Opin. Struct. Biol.* 7:377-87; Sippl *et al.*, 1996, *Structure* 4:15-19), "profile analysis" (Bowie *et al.*, 1991, *Science*, 253:164-70; Gribskov *et al.*, 1990, *Methods Enzymol.* 183:146-59; Gribskov *et al.*, 1987, *Proc. Nat. Acad. Sci. U.S.A.* 84:4355-58), and "evolutionary linkage" (See Holm *et al.*, *supra*, and Brenner *et al.*, *supra*).

Preferred IL-1ra-R polypeptide variants include glycosylation variants wherein the number and/or type of glycosylation sites have been altered compared to the amino acid sequence set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. In one embodiment, IL-1ra-R polypeptide variants comprise a greater or a lesser number of N-linked glycosylation sites than the amino acid sequence set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions that eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred IL-1ra-R variants include cysteine variants, wherein one or more cysteine residues are deleted or substituted with another amino acid (*e.g.*, serine) as compared to the amino acid sequence set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. Cysteine variants are useful when IL-1ra-R polypeptides must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

In other embodiments, related nucleic acid molecules comprise or consist of a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID

NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one amino acid insertion and wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID

5 NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one amino acid deletion and wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. Related nucleic acid molecules also comprise or consist of a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ

10 ID NO: 6, or SEQ ID NO: 36 wherein the polypeptide has a carboxyl- and/or amino-terminal truncation and further wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36. Related nucleic acid molecules also comprise or consist of a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 2,

15 SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, carboxyl-terminal truncations, and amino-terminal truncations and wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or

20 SEQ ID NO: 36.

In addition, the polypeptide comprising the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or other IL-1ra-R polypeptide, may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Heterologous

25 peptides and polypeptides include, but are not limited to: an epitope to allow for the detection and/or isolation of an IL-1ra-R fusion polypeptide; a transmembrane receptor protein or a portion thereof, such as an extracellular domain or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is

30 catalytically active; a polypeptide or peptide which promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases

stability, such as an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the polypeptide comprising the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or other IL-1ra-R polypeptide.

5 Fusions can be made either at the amino-terminus or at the carboxyl-terminus of the polypeptide comprising the amino acid sequence set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or other IL-1ra-R polypeptide. Fusions may be direct with no linker or adapter molecule or may be through a linker or adapter molecule. A linker or adapter molecule may  
10 be one or more amino acid residues, typically from about 20 to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein.

15 In a further embodiment of the invention, the polypeptide comprising the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or other IL-1ra-R polypeptide, is fused to one or more domains of an Fc region of human IgG. Antibodies comprise two functionally independent parts, a variable domain known as "Fab," that binds an antigen, and a constant  
20 domain known as "Fc," that is involved in effector functions such as complement activation and attack by phagocytic cells. An Fc has a long serum half-life, whereas an Fab is short-lived. Capon *et al.*, 1989, *Nature* 337:525-31. When constructed together with a therapeutic protein, an Fc domain can provide longer half-life or incorporate such functions as Fc receptor binding, protein A binding,  
25 complement fixation, and perhaps even placental transfer. *Id.* Table II summarizes the use of certain Fc fusions known in the art.

Table II

Fc Fusion with Therapeutic Proteins

Form of Fc	Fusion partner	Therapeutic implications	Reference
------------	----------------	--------------------------	-----------



IgG1	N-terminus of CD30-L	Hodgkin's disease; anaplastic lymphoma; T-cell leukemia	U.S. Patent No. 5,480,981
Murine Fcy2a	IL-10	anti-inflammatory; transplant rejection	Zheng <i>et al.</i> , 1995, <i>J. Immunol.</i> 154:5590-600
IgG1	TNF receptor	septic shock	Fisher <i>et al.</i> , 1996, <i>N. Engl. J. Med.</i> 334:1697-1702; Van Zee <i>et al.</i> , 1996, <i>J. Immunol.</i> 156:2221-30
IgG, IgA, IgM, or IgE (excluding the first domain)	TNF receptor	inflammation, autoimmune disorders	U.S. Patent No. 5,808,029
IgG1	CD4 receptor	AIDS	Capon <i>et al.</i> , 1989, <i>Nature</i> 337: 525-31
IgG1, IgG3	N-terminus of IL-2	anti-cancer, antiviral	Harvill <i>et al.</i> , 1995, <i>Immunotech.</i> 1:95-105
IgG1	C-terminus of OPG	osteoarthritis; bone density	WO 97/23614
IgG1	N-terminus of leptin	anti-obesity	PCT/US 97/23183, filed December 11, 1997
Human Ig C $\gamma$ 1	CTLA-4	autoimmune disorders	Linsley, 1991, <i>J. Exp. Med.</i> , 174:561-69

In one example, a human IgG hinge, CH2, and CH3 region may be fused at either the amino-terminus or carboxyl-terminus of the IL-1ra-R polypeptides using methods known to the skilled artisan. In another example, a human IgG

5 hinge, CH2, and CH3 region may be fused at either the amino-terminus or carboxyl-terminus of an IL-1ra-R polypeptide fragment (e.g., the predicted extracellular portion of IL-1ra-R polypeptide).

The resulting IL-1ra-R fusion polypeptide may be purified by use of a Protein A affinity column. Peptides and proteins fused to an Fc region have been

10 found to exhibit a substantially greater half-life *in vivo* than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion polypeptide. The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, such as therapeutic qualities, circulation time, or reduced aggregation.

Identity and similarity of related nucleic acid molecules and polypeptides are readily calculated by known methods. Such methods include, but are not limited to those described in *Computational Molecular Biology* (A.M. Lesk, ed., Oxford University Press 1988); *Biocomputing: Informatics and Genome Projects* (D.W. Smith, ed., Academic Press 1993); *Computer Analysis of Sequence Data* (Part 1, A.M. Griffin and H.G. Griffin, eds., Humana Press 1994); G. von Heinle, *Sequence Analysis in Molecular Biology* (Academic Press 1987); *Sequence Analysis Primer* (M. Gribskov and J. Devereux, eds., M. Stockton Press 1991); and Carillo *et al.*, 1988, *SIAM J. Applied Math.*, 48:1073.

Preferred methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, 1984, *Nucleic Acids Res.* 12:387; Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, and FASTA (Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (Altschul *et al.*, *BLAST Manual* (NCB 20 NLM NIH, Bethesda, MD); Altschul *et al.*, 1990, *supra*). The well-known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full-length sequences. Accordingly, in a preferred embodiment, the selected alignment method (GAP program) will result in an alignment that spans at least 50 contiguous amino acids of the claimed polypeptide.

For example, using the computer algorithm GAP (Genetics Computer 30 Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of

their respective amino acids (the "matched span," as determined by the algorithm). A gap opening penalty (which is calculated as 3X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 0.1X the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix is also used by the algorithm (see Dayhoff *et al.*, 5 *Atlas of Protein Sequence and Structure* (Supp. 3 1978)(PAM250 comparison matrix); Henikoff *et al.*, 1992, *Proc. Natl. Acad. Sci USA* 89:10915-19 (BLOSUM 62 comparison matrix)).

Preferred parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443-53;  
Comparison matrix: BLOSUM 62 (Henikoff *et al.*, *supra*);  
Gap Penalty: 12  
Gap Length Penalty: 4  
Threshold of Similarity: 0

20

The GAP program is useful with the above parameters. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

Preferred parameters for nucleic acid molecule sequence comparison include the following:

25

Algorithm: Needleman and Wunsch, *supra*;  
Comparison matrix: matches = +10, mismatch = 0  
Gap Penalty: 50  
Gap Length Penalty: 3

30

The GAP program is also useful with the above parameters. The aforementioned parameters are the default parameters for nucleic acid molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, and thresholds of similarity may be used, including those set forth in the Program Manual, Wisconsin Package, Version 9, September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA-to-DNA, protein-to-protein, protein-to-DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

#### Nucleic Acid Molecules

The nucleic acid molecules encoding a polypeptide comprising the amino acid sequence of an IL-1ra-R polypeptide can readily be obtained in a variety of ways including, without limitation, chemical synthesis, cDNA or genomic library screening, expression library screening, and/or PCR amplification of cDNA.

Recombinant DNA methods used herein are generally those set forth in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1989) and/or *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., Green Publishers Inc. and Wiley and Sons 1994). The invention provides for nucleic acid molecules as described herein and methods for obtaining such molecules.

Where a gene encoding the amino acid sequence of an IL-1ra-R polypeptide has been identified from one species, all or a portion of that gene may be used as a probe to identify orthologs or related genes from the same species. The probes or primers may be used to screen cDNA libraries from various tissue sources believed to express the IL-1ra-R polypeptide. In addition, part or all of a nucleic acid molecule having the sequence as set forth in any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35 may be used to screen a genomic library to identify and isolate a gene encoding the amino acid sequence

of an IL-1ra-R polypeptide. Typically, conditions of moderate or high stringency will be employed for screening to minimize the number of false positives obtained from the screening.

5 Nucleic acid molecules encoding the amino acid sequence of IL-1ra-R polypeptides may also be identified by expression cloning which employs the detection of positive clones based upon a property of the expressed protein. Typically, nucleic acid libraries are screened by the binding an antibody or other binding partner (*e.g.*, receptor or ligand) to cloned proteins that are expressed and displayed on a host cell surface. The antibody or binding partner is modified with  
10 a detectable label to identify those cells expressing the desired clone.

Recombinant expression techniques conducted in accordance with the descriptions set forth below may be followed to produce these polynucleotides and to express the encoded polypeptides. For example, by inserting a nucleic acid sequence that encodes the amino acid sequence of an IL-1ra-R polypeptide into an  
15 appropriate vector, one skilled in the art can readily produce large quantities of the desired nucleotide sequence. The sequences can then be used to generate detection probes or amplification primers. Alternatively, a polynucleotide encoding the amino acid sequence of an IL-1ra-R polypeptide can be inserted into an expression vector. By introducing the expression vector into an appropriate  
20 host, the encoded IL-1ra-R polypeptide may be produced in large amounts.

Another method for obtaining a suitable nucleic acid sequence is the polymerase chain reaction (PCR). In this method, cDNA is prepared from poly(A)+RNA or total RNA using the enzyme reverse transcriptase. Two primers, typically complementary to two separate regions of cDNA encoding the  
25 amino acid sequence of an IL-1ra-R polypeptide, are then added to the cDNA along with a polymerase such as *Taq* polymerase, and the polymerase amplifies the cDNA region between the two primers.

Another means of preparing a nucleic acid molecule encoding the amino acid sequence of an IL-1ra-R polypeptide is chemical synthesis using methods  
30 well known to the skilled artisan such as those described by Engels *et al.*, 1989, *Angew. Chem. Intl. Ed.* 28:716-34. These methods include, *inter alia*, the

phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding the amino acid sequence of an IL-1ra-R polypeptide will be several  
5 hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments can then be ligated together to form the full-length nucleotide sequence of an IL-1ra-R gene. Usually, the DNA fragment encoding the amino-terminus of the polypeptide will have an ATG, which encodes a methionine residue. This  
10 methionine may or may not be present on the mature form of the IL-1ra-R polypeptide, depending on whether the polypeptide produced in the host cell is designed to be secreted from that cell. Other methods known to the skilled artisan may be used as well.

In certain embodiments, nucleic acid variants contain codons which have  
15 been altered for optimal expression of an IL-1ra-R polypeptide in a given host cell. Particular codon alterations will depend upon the IL-1ra-R polypeptide and host cell selected for expression. Such "codon optimization" can be carried out by a variety of methods, for example, by selecting codons which are preferred for use in highly expressed genes in a given host cell. Computer algorithms which  
20 incorporate codon frequency tables such as "Eco\_high.Cod" for codon preference of highly expressed bacterial genes may be used and are provided by the University of Wisconsin Package Version 9.0 (Genetics Computer Group, Madison, WI). Other useful codon frequency tables include "Celegans\_high.cod," "Celegans\_low.cod," "Drosophila\_high.cod,"  
25 "Human\_high.cod," "Maize\_high.cod," and "Yeast\_high.cod."

In some cases, it may be desirable to prepare nucleic acid molecules encoding IL-1ra-R polypeptide variants. Nucleic acid molecules encoding variants may be produced using site directed mutagenesis, PCR amplification, or other appropriate methods, where the primer(s) have the desired point mutations  
30 (see Sambrook *et al.*, *supra*, and Ausubel *et al.*, *supra*, for descriptions of mutagenesis techniques). Chemical synthesis using methods described by Engels

*et al.*, *supra*, may also be used to prepare such variants. Other methods known to the skilled artisan may be used as well.

### Vectors and Host Cells

5           A nucleic acid molecule encoding the amino acid sequence of an IL-1ra-R polypeptide is inserted into an appropriate expression vector using standard ligation techniques. The vector is typically selected to be functional in the particular host cell employed (*i.e.*, the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can  
10       occur). A nucleic acid molecule encoding the amino acid sequence of an IL-1ra-R polypeptide may be amplified/expressed in prokaryotic, yeast, insect (baculovirus systems) and/or eukaryotic host cells. Selection of the host cell will depend in part on whether an IL-1ra-R polypeptide is to be post-translationally modified (*e.g.*, glycosylated and/or phosphorylated). If so, yeast, insect, or mammalian host  
15       cells are preferable. For a review of expression vectors, *see Meth. Enz.*, vol. 185 (D.V. Goeddel, ed., Academic Press 1990).

          Typically, expression vectors used in any of the host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as "flanking  
20       sequences" in certain embodiments will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a  
25       polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these sequences is discussed below.

          Optionally, the vector may contain a "tag"-encoding sequence, *i.e.*, an oligonucleotide molecule located at the 5' or 3' end of the IL-1ra-R polypeptide  
30       coding sequence; the oligonucleotide sequence encodes polyHis (such as hexaHis), or another "tag" such as FLAG, HA (hemagglutinin influenza virus), or

*myc* for which commercially available antibodies exist. This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of the IL-1ra-R polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using  
5 antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified IL-1ra-R polypeptide by various means such as using certain peptidases for cleavage.

Flanking sequences may be homologous (*i.e.*, from the same species and/or strain as the host cell), heterologous (*i.e.*, from a species other than the host  
10 cell species or strain), hybrid (*i.e.*, a combination of flanking sequences from more than one source), or synthetic, or the flanking sequences may be native sequences which normally function to regulate IL-1ra-R polypeptide expression. As such, the source of a flanking sequence may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking  
15 sequence is functional in, and can be activated by, the host cell machinery.

Flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, flanking sequences useful herein – other than the IL-1ra-R gene flanking sequences – will have been previously identified by mapping and/or by restriction endonuclease digestion and  
20 can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of a flanking sequence may be known. Here, the flanking sequence may be synthesized using the methods described herein for nucleic acid synthesis or cloning.

Where all or only a portion of the flanking sequence is known, it may be  
25 obtained using PCR and/or by screening a genomic library with a suitable oligonucleotide and/or flanking sequence fragment from the same or another species. Where the flanking sequence is not known, a fragment of DNA containing a flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes.  
30 Isolation may be accomplished by restriction endonuclease digestion to produce the proper DNA fragment followed by isolation using agarose gel purification,



Qiagen® column chromatography (Chatsworth, CA), or other methods known to the skilled artisan. The selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

An origin of replication is typically a part of those prokaryotic expression  
5 vectors purchased commercially, and the origin aids in the amplification of the vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for the optimal expression of an IL-1ra-R polypeptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector.  
10 For example, the origin of replication from the plasmid pBR322 (New England Biolabs, Beverly, MA) is suitable for most gram-negative bacteria and various origins (e.g., SV40, polyoma, adenovirus, vesicular stomatitis virus (VSV), or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed  
15 for mammalian expression vectors (for example, the SV40 origin is often used only because it contains the early promoter).

A transcription termination sequence is typically located 3' of the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment  
20 followed by a poly-T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis such as those described herein.

A selectable marker gene element encodes a protein necessary for the  
25 survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells; (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex media. Preferred selectable markers are the  
30 kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. A neomycin resistance gene may also be used for selection in

prokaryotic and eukaryotic host cells.

Other selection genes may be used to amplify the gene that will be expressed. Amplification is the process wherein genes that are in greater demand for the production of a protein critical for growth are reiterated in tandem within  
5 the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR) and thymidine kinase. The mammalian cell transformants are placed under selection pressure wherein only the transformants are uniquely adapted to survive by virtue of the selection gene present in the vector. Selection pressure is  
10 imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to the amplification of both the selection gene and the DNA that encodes an IL-1ra-R polypeptide. As a result, increased quantities of IL-1ra-R polypeptide are synthesized from the amplified DNA.

15 A ribosome binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3' to the promoter and 5' to the coding sequence of an IL-1ra-R polypeptide to be expressed. The Shine-Dalgarno sequence is varied but is typically a polypurine (*i.e.*, having a  
20 high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized using methods set forth herein and used in a prokaryotic vector.

A leader, or signal, sequence may be used to direct an IL-1ra-R polypeptide out of the host cell. Typically, a nucleotide sequence encoding the  
25 signal sequence is positioned in the coding region of an IL-1ra-R nucleic acid molecule, or directly at the 5' end of an IL-1ra-R polypeptide coding region. Many signal sequences have been identified, and any of those that are functional in the selected host cell may be used in conjunction with an IL-1ra-R nucleic acid molecule. Therefore, a signal sequence may be homologous (naturally occurring)  
30 or heterologous to the IL-1ra-R nucleic acid molecule. Additionally, a signal sequence may be chemically synthesized using methods described herein. In most

cases, the secretion of an IL-1ra-R polypeptide from the host cell via the presence of a signal peptide will result in the removal of the signal peptide from the secreted IL-1ra-R polypeptide. The signal sequence may be a component of the vector, or it may be a part of an IL-1ra-R nucleic acid molecule that is inserted  
5 into the vector.

Included within the scope of this invention is the use of either a nucleotide sequence encoding a native IL-1ra-R polypeptide signal sequence joined to an IL-1ra-R polypeptide coding region or a nucleotide sequence encoding a heterologous signal sequence joined to an IL-1ra-R polypeptide coding region.  
10 The heterologous signal sequence selected should be one that is recognized and processed, *i.e.*, cleaved by a signal peptidase, by the host cell. For prokaryotic host cells that do not recognize and process the native IL-1ra-R polypeptide signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, or  
15 heat-stable enterotoxin II leaders. For yeast secretion, the native IL-1ra-R polypeptide signal sequence may be substituted by the yeast invertase, alpha factor, or acid phosphatase leaders. In mammalian cell expression the native signal sequence is satisfactory, although other mammalian signal sequences may be suitable.

In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various presequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add pro-sequences, which also may affect glycosylation. The final protein product may have, in the -1 position (relative to  
25 the first amino acid of the mature protein) one or more additional amino acids incident to expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the amino-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired  
30 IL-1ra-R polypeptide, if the enzyme cuts at such area within the mature polypeptide.

In many cases, transcription of a nucleic acid molecule is increased by the presence of one or more introns in the vector; this is particularly true where a polypeptide is produced in eukaryotic host cells, especially mammalian host cells. The introns used may be naturally occurring within the IL-1ra-R gene especially  
5 where the gene used is a full-length genomic sequence or a fragment thereof. Where the intron is not naturally occurring within the gene (as for most cDNAs), the intron may be obtained from another source. The position of the intron with respect to flanking sequences and the IL-1ra-R gene is generally important, as the intron must be transcribed to be effective. Thus, when an IL-1ra-R cDNA  
10 molecule is being transcribed, the preferred position for the intron is 3' to the transcription start site and 5' to the poly-A transcription termination sequence. Preferably, the intron or introns will be located on one side or the other (*i.e.*, 5' or 3') of the cDNA such that it does not interrupt the coding sequence. Any intron from any source, including viral, prokaryotic and eukaryotic (plant or animal)  
15 organisms, may be used to practice this invention, provided that it is compatible with the host cell into which it is inserted. Also included herein are synthetic introns. Optionally, more than one intron may be used in the vector.

The expression and cloning vectors of the present invention will typically contain a promoter that is recognized by the host organism and operably linked to  
20 the molecule encoding the IL-1ra-R polypeptide. Promoters are untranscribed sequences located upstream (*i.e.*, 5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription of the structural gene. Promoters are conventionally grouped into one of two classes: inducible promoters and constitutive promoters. Inducible promoters initiate  
25 increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, initiate continual gene product production; that is, there is little or no control over gene expression. A large number of promoters, recognized by a variety of potential  
30 host cells, are well known. A suitable promoter is operably linked to the DNA encoding IL-1ra-R polypeptide by removing the promoter from the source DNA

by restriction enzyme digestion and inserting the desired promoter sequence into the vector. The native IL-1ra-R promoter sequence may be used to direct amplification and/or expression of an IL-1ra-R nucleic acid molecule. A heterologous promoter is preferred, however, if it permits greater transcription and higher yields of the expressed protein as compared to the native promoter, and if it is compatible with the host cell system that has been selected for use.

Promoters suitable for use with prokaryotic hosts include the beta-lactamase and lactose promoter systems; alkaline phosphatase; a tryptophan (trp) promoter system; and hybrid promoters such as the tac promoter. Other known bacterial promoters are also suitable. Their sequences have been published, thereby enabling one skilled in the art to ligate them to the desired DNA sequence, using linkers or adapters as needed to supply any useful restriction sites.

Suitable promoters for use with yeast hosts are also well known in the art. Yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, retroviruses, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, for example, heat-shock promoters and the actin promoter.

Additional promoters which may be of interest in controlling IL-1ra-R gene expression include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-10); the CMV promoter; the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, 1980, *Cell* 22:787-97); the herpes thymidine kinase promoter (Wagner *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1444-45); the regulatory sequences of the metallothioneine gene (Brinster *et al.*, 1982, *Nature* 296:39-42); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Kamaroff *et al.*, 1978, *Proc. Natl. Acad. Sci. U.S.A.*, 75:3727-31); or the tac promoter (DeBoer *et al.*, 1983, *Proc. Natl. Acad. Sci. U.S.A.*, 80:21-25). Also of

interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, *Cell* 38:639-46; Ornitz *et al.*, 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409 (1986); MacDonald, 1987, *Hepatology* 7:425-515); the insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-22); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, *Cell* 38:647-58; Adames *et al.*, 1985, *Nature* 318:533-38; Alexander *et al.*, 1987, *Mol. Cell. Biol.*, 7:1436-44); the mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, *Cell* 45:485-95); the albumin gene control region which is active in liver (Pinkert *et al.*, 1987, *Genes and Devel.* 1:268-76); the alpha-feto-protein gene control region which is active in liver (Krumlauf *et al.*, 1985, *Mol. Cell. Biol.*, 5:1639-48; Hammer *et al.*, 1987, *Science* 235:53-58); the alpha 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, 1987, *Genes and Devel.* 1:161-71); the beta-globin gene control region which is active in myeloid cells (Mogam *et al.*, 1985, *Nature* 315:338-40; Kollias *et al.*, 1986, *Cell* 46:89-94); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, *Cell* 48:703-12); the myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-86); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, 1986, *Science* 234:1372-78).

An enhancer sequence may be inserted into the vector to increase the transcription of a DNA encoding an IL-1ra-R polypeptide of the present invention by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent. They have been found 5' and 3' to the transcription unit. Several enhancer sequences available from mammalian genes are known (*e.g.*, globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus will be used. The

SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be spliced into the vector at a position 5' or 3' to an IL-1ra-R nucleic acid molecule, it is typically  
5 located at a site 5' from the promoter.

Expression vectors of the invention may be constructed from a starting vector such as a commercially available vector. Such vectors may or may not contain all of the desired flanking sequences. Where one or more of the flanking sequences described herein are not already present in the vector, they may be  
10 individually obtained and ligated into the vector. Methods used for obtaining each of the flanking sequences are well known to one skilled in the art.

Preferred vectors for practicing this invention are those which are compatible with bacterial, insect, and mammalian host cells. Such vectors include, *inter alia*, pCRII, pCR3, and pcDNA3.1 (Invitrogen, San Diego, CA),  
15 pBSII (Stratagene, La Jolla, CA), pET15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII, Invitrogen), pDSR-alpha (PCT Pub. No. WO 90/14363) and pFastBacDual (Gibco-BRL, Grand Island, NY).

Additional suitable vectors include, but are not limited to, cosmids,  
20 plasmids, or modified viruses, but it will be appreciated that the vector system must be compatible with the selected host cell. Such vectors include, but are not limited to plasmids such as Bluescript® plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems, La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (*e.g.*, TOPO™ TA  
25 Cloning® Kit, PCR2.1® plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA).

After the vector has been constructed and a nucleic acid molecule encoding an IL-1ra-R polypeptide has been inserted into the proper site of the  
30 vector, the completed vector may be inserted into a suitable host cell for

amplification and/or polypeptide expression. The transformation of an expression vector for an IL-1ra-R polypeptide into a selected host cell may be accomplished by well known methods including methods such as transfection, infection, calcium chloride, electroporation, microinjection, lipofection, DEAE-dextran  
5 method, or other known techniques. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic  
10 host cells (such as a yeast, insect, or vertebrate cell). The host cell, when cultured under appropriate conditions, synthesizes an IL-1ra-R polypeptide which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). The selection of an appropriate host cell will depend upon various factors, such as  
15 desired expression levels, polypeptide modifications that are desirable or necessary for activity (such as glycosylation or phosphorylation) and ease of folding into a biologically active molecule.

A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC), Manassas, VA.  
20 Examples include, but are not limited to, mammalian cells, such as Chinese hamster ovary cells (CHO), CHO DHFR(-) cells (Urlaub *et al.*, 1980, *Proc. Natl. Acad. Sci. U.S.A.* 97:4216-20), human embryonic kidney (HEK) 293 or 293T cells, or 3T3 cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening, product production, and  
25 purification are known in the art. Other suitable mammalian cell lines, are the monkey COS-1 and COS-7 cell lines, and the CV-1 cell line. Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, as well as primary explants, are also suitable. Candidate  
30 cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but



are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines. Each of these cell lines is known by and available to those skilled in the art of protein expression.

5 Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101, DH5 $\alpha$ , DH10, and MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and the like may also be employed in this method.

10 Many strains of yeast cells known to those skilled in the art are also available as host cells for the expression of the polypeptides of the present invention. Preferred yeast cells include, for example, *Saccharomyces cerevisiae* and *Pichia pastoris*.

15 Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described, for example, in Kitts *et al.*, 1993, *Biotechniques*, 14:810-17; Lucklow, 1993, *Curr. Opin. Biotechnol.* 4:564-72; and Lucklow *et al.*, 1993, *J. Virol.*, 67:4566-79. Preferred insect cells are Sf-9 and Hi5 (Invitrogen).

20 One may also use transgenic animals to express glycosylated IL-1ra-R polypeptides. For example, one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain the present glycosylated polypeptide in the animal milk. One may also use plants to produce IL-1ra-R polypeptides, however, in general, the glycosylation occurring in plants is different from that produced in mammalian cells, and may result in a glycosylated product which is  
25 not suitable for human therapeutic use.

#### Polypeptide Production

Host cells comprising an IL-1ra-R polypeptide expression vector may be cultured using standard media well known to the skilled artisan. The media will  
30 usually contain all nutrients necessary for the growth and survival of the cells. Suitable media for culturing *E. coli* cells include, for example, Luria Broth (LB)

and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells include Roswell Park Memorial Institute medium 1640 (RPMI 1640), Minimal Essential Medium (MEM) and/or Dulbecco's Modified Eagle Medium (DMEM), all of which may be supplemented with serum and/or growth factors as necessary for the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin hydrolysate, and/or fetal calf serum as necessary.

Typically, an antibiotic or other compound useful for selective growth of transfected or transformed cells is added as a supplement to the media. The compound to be used will be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin. Other compounds for selective growth include ampicillin, tetracycline, and neomycin.

The amount of an IL-1ra-R polypeptide produced by a host cell can be evaluated using standard methods known in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, High Performance Liquid Chromatography (HPLC) separation, immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

If an IL-1ra-R polypeptide has been designed to be secreted from the host cells, the majority of polypeptide may be found in the cell culture medium. If however, the IL-1ra-R polypeptide is not secreted from the host cells, it will be present in the cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol (for gram-negative bacteria host cells).

For an IL-1ra-R polypeptide situated in the host cell cytoplasm and/or nucleus (for eukaryotic host cells) or in the cytosol (for bacterial host cells), the intracellular material (including inclusion bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication

followed by centrifugation.

If an IL-1ra-R polypeptide has formed inclusion bodies in the cytosol, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet  
5 material can then be treated at pH extremes or with a chaotropic agent such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. The solubilized IL-1ra-R polypeptide can then be analyzed  
10 using gel electrophoresis, immunoprecipitation, or the like. If it is desired to isolate the IL-1ra-R polypeptide, isolation may be accomplished using standard methods such as those described herein and in Marston *et al.*, 1990, *Meth. Enz.*, 182:264-75.

In some cases, an IL-1ra-R polypeptide may not be biologically active  
15 upon isolation. Various methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages can be used to restore biological activity. Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for  
20 inclusion body solubilization, but usually the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In most cases the refolding/oxidation solution will also contain a reducing agent or the reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in  
25 the formation of the protein's cysteine bridges. Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/dithiane DTT, and 2-2-mercaptoethanol(bME)/dithio-b(ME). In many instances, a cosolvent may be used or may be needed to increase the efficiency of the refolding, and the more  
30 common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, arginine and the like.

If inclusion bodies are not formed to a significant degree upon expression of an IL-1ra-R polypeptide, then the polypeptide will be found primarily in the supernatant after centrifugation of the cell homogenate. The polypeptide may be further isolated from the supernatant using methods such as those described  
5 herein.

The purification of an IL-1ra-R polypeptide from solution can be accomplished using a variety of techniques. If the polypeptide has been synthesized such that it contains a tag such as Hexahistidine (IL-1ra-R polypeptide/hexaHis) or other small peptide such as FLAG (Eastman Kodak Co.,  
10 New Haven, CT) or *myc* (Invitrogen, Carlsbad, CA) at either its carboxyl- or amino-terminus, it may be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag.

For example, polyhistidine binds with great affinity and specificity to  
15 nickel. Thus, an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of IL-1ra-R polypeptide/polyHis. See, e.g., *Current Protocols in Molecular Biology* § 10.11.8 (Ausubel *et al.*, eds., Green Publishers Inc. and Wiley and Sons 1993).

Additionally, IL-1RA-R polypeptides may be purified through the use of a  
20 monoclonal antibody that is capable of specifically recognizing and binding to an IL-1ra-R polypeptide.

Other suitable procedures for purification include, without limitation, affinity chromatography, immunoaffinity chromatography, ion exchange chromatography, molecular sieve chromatography, HPLC, electrophoresis  
25 (including native gel electrophoresis) followed by gel elution, and preparative isoelectric focusing ("Isoprime" machine/technique, Hoefer Scientific, San Francisco, CA). In some cases, two or more purification techniques may be combined to achieve increased purity.

IL-1ra-R polypeptides may also be prepared by chemical synthesis  
30 methods (such as solid phase peptide synthesis) using techniques known in the art such as those set forth by Merrifield *et al.*, 1963, *J. Am. Chem. Soc.* 85:2149;

Houghten *et al.*, 1985, *Proc Natl Acad. Sci. USA* 82:5132; and Stewart and Young, *Solid Phase Peptide Synthesis* (Pierce Chemical Co. 1984). Such polypeptides may be synthesized with or without a methionine on the amino-terminus. Chemically synthesized IL-1ra-R polypeptides may be oxidized using  
5 methods set forth in these references to form disulfide bridges. Chemically synthesized IL-1ra-R polypeptides are expected to have comparable biological activity to the corresponding IL-1ra-R polypeptides produced recombinantly or purified from natural sources, and thus may be used interchangeably with a recombinant or natural IL-1ra-R polypeptide.

10 Another means of obtaining IL-1ra-R polypeptide is via purification from biological samples such as source tissues and/or fluids in which the IL-1ra-R polypeptide is naturally found. Such purification can be conducted using methods for protein purification as described herein. The presence of the IL-1ra-R polypeptide during purification may be monitored, for example, using an antibody  
15 prepared against recombinantly produced IL-1ra-R polypeptide or peptide fragments thereof.

A number of additional methods for producing nucleic acids and polypeptides are known in the art, and the methods can be used to produce polypeptides having specificity for IL-1ra-R polypeptide. *See, e.g., Roberts et al.*,  
20 1997, *Proc. Natl. Acad. Sci. U.S.A.* 94:12297-303, which describes the production of fusion proteins between an mRNA and its encoded peptide. *See also, Roberts*, 1999, *Curr. Opin. Chem. Biol.* 3:268-73. Additionally, U.S. Patent No. 5,824,469 describes methods for obtaining oligonucleotides capable of carrying out a specific biological function. The procedure involves generating a heterogeneous  
25 pool of oligonucleotides, each having a 5' randomized sequence, a central preselected sequence, and a 3' randomized sequence. The resulting heterogeneous pool is introduced into a population of cells that do not exhibit the desired biological function. Subpopulations of the cells are then screened for those that exhibit a predetermined biological function. From that subpopulation,  
30 oligonucleotides capable of carrying out the desired biological function are isolated.

U.S. Patent Nos. 5,763,192; 5,814,476; 5,723,323; and 5,817,483 describe processes for producing peptides or polypeptides. This is done by producing stochastic genes or fragments thereof, and then introducing these genes into host cells which produce one or more proteins encoded by the stochastic genes. The  
5 host cells are then screened to identify those clones producing peptides or polypeptides having the desired activity.

Another method for producing peptides or polypeptides is described in PCT/US98/20094 (WO99/15650) filed by Athersys, Inc. Known as "Random Activation of Gene Expression for Gene Discovery" (RAGE-GD), the process  
10 involves the activation of endogenous gene expression or over-expression of a gene by *in situ* recombination methods. For example, expression of an endogenous gene is activated or increased by integrating a regulatory sequence into the target cell which is capable of activating expression of the gene by non-homologous or illegitimate recombination. The target DNA is first subjected to  
15 radiation, and a genetic promoter inserted. The promoter eventually locates a break at the front of a gene, initiating transcription of the gene. This results in expression of the desired peptide or polypeptide.

It will be appreciated that these methods can also be used to create comprehensive IL-1ra-R polypeptide expression libraries, which can subsequently  
20 be used for high throughput phenotypic screening in a variety of assays, such as biochemical assays, cellular assays, and whole organism assays (e.g., plant, mouse, etc.).

### Synthesis

25 It will be appreciated by those skilled in the art that the nucleic acid and polypeptide molecules described herein may be produced by recombinant and other means.

### Selective Binding Agents

30 The term "selective binding agent" refers to a molecule that has specificity for one or more IL-1ra-R polypeptides. Suitable selective binding agents include,

but are not limited to, antibodies and derivatives thereof, polypeptides, and small molecules. Suitable selective binding agents may be prepared using methods known in the art. An exemplary IL-1RA-R polypeptide selective binding agent of the present invention is capable of binding a certain portion of the IL-1RA-R polypeptide thereby inhibiting the binding of the polypeptide to an IL-1ra-R polypeptide receptor.

Selective binding agents such as antibodies and antibody fragments that bind IL-1ra-R polypeptides are within the scope of the present invention. The antibodies may be polyclonal including monospecific polyclonal; monoclonal (MAbs); recombinant; chimeric; humanized, such as CDR-grafted; human; single chain; and/or bispecific; as well as fragments; variants; or derivatives thereof. Antibody fragments include those portions of the antibody that bind to an epitope on the IL-1RA-R polypeptide. Examples of such fragments include Fab and F(ab') fragments generated by enzymatic cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions.

Polyclonal antibodies directed toward an IL-1ra-R polypeptide generally are produced in animals (e.g., rabbits or mice) by means of multiple subcutaneous or intraperitoneal injections of IL-1ra-R polypeptide and an adjuvant. It may be useful to conjugate an IL-1ra-R polypeptide to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet hemocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-IL-1ra-R antibody titer.

Monoclonal antibodies directed toward IL-1ra-R polypeptides are produced using any method that provides for the production of antibody molecules by continuous cell lines in culture. Examples of suitable methods for preparing monoclonal antibodies include the hybridoma methods of Kohler *et al.*, 1975, *Nature* 256:495-97 and the human B-cell hybridoma method (Kozbor,

1984, *J. Immunol.* 133:3001; Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications* 51-63 (Marcel Dekker, Inc., 1987). Also provided by the invention are hybridoma cell lines that produce monoclonal antibodies reactive with IL-1ra-R polypeptides.

5           Monoclonal antibodies of the invention may be modified for use as therapeutics. One embodiment is a "chimeric" antibody in which a portion of the heavy (H) and/or light (L) chain is identical with or homologous to a corresponding sequence in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to a corresponding sequence in  
10 antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies, so long as they exhibit the desired biological activity. See U.S. Patent No. 4,816,567; Morrison *et al.*, 1985, *Proc. Natl. Acad. Sci.* 81:6851-55.

15           In another embodiment, a monoclonal antibody of the invention is a "humanized" antibody. Methods for humanizing non-human antibodies are well known in the art. See U.S. Patent Nos. 5,585,089 and 5,693,762. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. Humanization can be performed, for example, using  
20 methods described in the art (Jones *et al.*, 1986, *Nature* 321:522-25; Riechmann *et al.*, 1998, *Nature* 332:323-27; Verhoeven *et al.*, 1988, *Science* 239:1534-36), by substituting at least a portion of a rodent complementarity-determining region (CDR) for the corresponding regions of a human antibody.

          Also encompassed by the invention are human antibodies that bind IL-1ra-  
25 R polypeptides. Using transgenic animals (*e.g.*, mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production such antibodies are produced by immunization with an IL-1ra-R polypeptide antigen (*i.e.*, having at least 6 contiguous amino acids), optionally conjugated to a carrier. See, *e.g.*, Jakobovits *et al.*, 1993, *Proc. Natl.*  
30 *Acad. Sci.* 90:2551-55; Jakobovits *et al.*, 1993, *Nature* 362:255-58; Bruggermann *et al.*, 1993, *Year in Immuno.* 7:33. In one method, such transgenic animals are



produced by incapacitating the endogenous loci encoding the heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain proteins into the genome thereof. Partially modified animals, that is those having less than the full complement of modifications, are then cross-bred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals produce antibodies with human (rather than, *e.g.*, murine) amino acid sequences, including variable regions which are immunospecific for these antigens. *See* PCT App. Nos. PCT/US96/05928 and PCT/US93/06926. Additional methods are described in U.S. Patent No. 5,545,807, PCT App. Nos. PCT/US91/245 and PCT/GB89/01207, and in European Patent Nos. 546073B1 and 546073A1. Human antibodies can also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

In an alternative embodiment, human antibodies can also be produced from phage-display libraries (Hoogenboom *et al.*, 1991, *J. Mol. Biol.* 227:381; Marks *et al.*, 1991, *J. Mol. Biol.* 222:581). These processes mimic immune selection through the display of antibody repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to an antigen of choice. One such technique is described in PCT App. No. PCT/US98/17364, which describes the isolation of high affinity and functional agonistic antibodies for MPL- and msk- receptors using such an approach.

Chimeric, CDR grafted, and humanized antibodies are typically produced by recombinant methods. Nucleic acids encoding the antibodies are introduced into host cells and expressed using materials and procedures described herein. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO cells. Monoclonal (*e.g.*, human) antibodies may be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

The anti-IL-1ra-R antibodies of the invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays (Sola, *Monoclonal Antibodies:*

*A Manual of Techniques* 147-158 (CRC Press, Inc., 1987)) for the detection and quantitation of IL-1ra-R polypeptides. The antibodies will bind IL-1ra-R polypeptides with an affinity that is appropriate for the assay method being employed.

5 For diagnostic applications, in certain embodiments, anti-IL-1ra-R antibodies may be labeled with a detectable moiety. The detectable moiety can be any one that is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{125}\text{I}$ ,  $^{99}\text{Tc}$ ,  $^{111}\text{In}$ , or  $^{67}\text{Ga}$ ; a fluorescent or chemiluminescent compound, 10 such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase,  $\beta$ -galactosidase, or horseradish peroxidase (Bayer, *et al.*, 1990, *Meth. Enz.* 184:138-63).

Competitive binding assays rely on the ability of a labeled standard (*e.g.*, an IL-1ra-R polypeptide, or an immunologically reactive portion thereof) to 15 compete with the test sample analyte (an IL-1ra-R polypeptide) for binding with a limited amount of anti-IL-1ra-R antibody. The amount of an IL-1ra-R polypeptide in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies typically are insolubilized before or 20 after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays typically involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be 25 detected and/or quantitated. In a sandwich assay, the test sample analyte is typically bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. *See, e.g.*, U.S. Patent No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be 30 measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assays). For example, one type of sandwich assay is an

enzyme-linked immunosorbent assay (ELISA), in which case the detectable moiety is an enzyme.

The selective binding agents, including anti-IL-1ra-R antibodies, are also useful for *in vivo* imaging. An antibody labeled with a detectable moiety may be administered to an animal, preferably into the bloodstream, and the presence and location of the labeled antibody in the host assayed. The antibody may be labeled with any moiety that is detectable in an animal, whether by nuclear magnetic resonance, radiology, or other detection means known in the art.

Selective binding agents of the invention, including antibodies, may be used as therapeutics. These therapeutic agents are generally agonists or antagonists, in that they either enhance or reduce, respectively, at least one of the biological activities of an IL-1ra-R polypeptide. In one embodiment, antagonist antibodies of the invention are antibodies or binding fragments thereof which are capable of specifically binding to an IL-1ra-R polypeptide and which are capable of inhibiting or eliminating the functional activity of an IL-1ra-R polypeptide *in vivo* or *in vitro*. In preferred embodiments, the selective binding agent, *e.g.*, an antagonist antibody, will inhibit the functional activity of an IL-1ra-R polypeptide by at least about 50%, and preferably by at least about 80%. In another embodiment, the selective binding agent may be an anti-IL-1ra-R polypeptide antibody that is capable of interacting with an IL-1ra-R polypeptide binding partner (a ligand or receptor) thereby inhibiting or eliminating IL-1ra-R polypeptide activity *in vitro* or *in vivo*. Selective binding agents, including agonist and antagonist anti-IL-1ra-R polypeptide antibodies, are identified by screening assays that are well known in the art.

The invention also relates to a kit comprising IL-1ra-R selective binding agents (such as antibodies) and other reagents useful for detecting IL-1ra-R polypeptide levels in biological samples. Such reagents may include a detectable label, blocking serum, positive and negative control samples, and detection reagents.

30

#### Microarrays

It will be appreciated that DNA microarray technology can be utilized in accordance with the present invention. DNA microarrays are miniature, high-density arrays of nucleic acids positioned on a solid support, such as glass. Each cell or element within the array contains numerous copies of a single nucleic acid species that acts as a target for hybridization with a complementary nucleic acid sequence (e.g., mRNA). In expression profiling using DNA microarray technology, mRNA is first extracted from a cell or tissue sample and then converted enzymatically to fluorescently labeled cDNA. This material is hybridized to the microarray and unbound cDNA is removed by washing. The expression of discrete genes represented on the array is then visualized by quantitating the amount of labeled cDNA that is specifically bound to each target nucleic acid molecule. In this way, the expression of thousands of genes can be quantitated in a high throughput, parallel manner from a single sample of biological material.

This high throughput expression profiling has a broad range of applications with respect to the IL-1ra-R molecules of the invention, including, but not limited to: the identification and validation of IL-1ra-R disease-related genes as targets for therapeutics; molecular toxicology of related IL-1ra-R molecules and inhibitors thereof; stratification of populations and generation of surrogate markers for clinical trials; and enhancing related IL-1ra-R polypeptide small molecule drug discovery by aiding in the identification of selective compounds in high throughput screens.

#### Chemical Derivatives

Chemically modified derivatives of IL-1ra-R polypeptides may be prepared by one skilled in the art, given the disclosures described herein. IL-1ra-R polypeptide derivatives are modified in a manner that is different – either in the type or location of the molecules naturally attached to the polypeptide. Derivatives may include molecules formed by the deletion of one or more naturally-attached chemical groups. The polypeptide comprising the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID

NO: 36, or other IL-1ra-R polypeptide, may be modified by the covalent attachment of one or more polymers. For example, the polymer selected is typically water-soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment.

- 5 Included within the scope of suitable polymers is a mixture of polymers. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable.

The polymers each may be of any molecular weight and may be branched or unbranched. The polymers each typically have an average molecular weight of  
10 between about 2 kDa to about 100 kDa (the term "about" indicating that in preparations of a water-soluble polymer, some molecules will weigh more, some less, than the stated molecular weight). The average molecular weight of each polymer is preferably between about 5 kDa and about 50 kDa, more preferably between about 12 kDa and about 40 kDa and most preferably between about 20  
15 kDa and about 35 kDa.

Suitable water-soluble polymers or mixtures thereof include, but are not limited to, N-linked or O-linked carbohydrates, sugars, phosphates, polyethylene glycol (PEG) (including the forms of PEG that have been used to derivatize proteins, including mono-(C<sub>1</sub>-C<sub>10</sub>), alkoxy-, or aryloxy-polyethylene glycol),  
20 monomethoxy-polyethylene glycol, dextran (such as low molecular weight dextran of, for example, about 6 kD), cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), and polyvinyl alcohol. Also  
25 encompassed by the present invention are bifunctional crosslinking molecules which may be used to prepare covalently attached IL-1ra-R polypeptide multimers.

In general, chemical derivatization may be performed under any suitable condition used to react a protein with an activated polymer molecule. Methods for  
30 preparing chemical derivatives of polypeptides will generally comprise the steps of: (a) reacting the polypeptide with the activated polymer molecule (such as a

reactive ester or aldehyde derivative of the polymer molecule) under conditions whereby the polypeptide comprising the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or other IL-1ra-R polypeptide, becomes attached to one or more polymer molecules, and (b)

5 obtaining the reaction products. The optimal reaction conditions will be determined based on known parameters and the desired result. For example, the larger the ratio of polymer molecules to protein, the greater the percentage of attached polymer molecule. In one embodiment, the IL-1ra-R polypeptide derivative may have a single polymer molecule moiety at the amino-terminus.

10 See, e.g., U.S. Patent No. 5,234,784.

The pegylation of a polypeptide may be specifically carried out using any of the pegylation reactions known in the art. Such reactions are described, for example, in the following references: Francis *et al.*, 1992, *Focus on Growth Factors* 3:4-10; European Patent Nos. 0154316 and 0401384; and U.S. Patent No.

15 4,179,337. For example, pegylation may be carried out via an acylation reaction or an alkylation reaction with a reactive polyethylene glycol molecule (or an analogous reactive water-soluble polymer) as described herein. For the acylation reactions, a selected polymer should have a single reactive ester group. For reductive alkylation, a selected polymer should have a single reactive aldehyde

20 group. A reactive aldehyde is, for example, polyethylene glycol propionaldehyde, which is water stable, or mono C<sub>1</sub>-C<sub>10</sub> alkoxy or aryloxy derivatives thereof (see U.S. Patent No. 5,252,714).

In another embodiment, IL-1ra-R polypeptides may be chemically coupled to biotin. The biotin/IL-1ra-R polypeptide molecules are then allowed to bind to

25 avidin, resulting in tetravalent avidin/biotin/IL-1ra-R polypeptide molecules. IL-1ra-R polypeptides may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugates precipitated with anti-DNP or anti-TNP-IgM to form decameric conjugates with a valency of 10.

Generally, conditions that may be alleviated or modulated by the

30 administration of the present IL-1ra-R polypeptide derivatives include those described herein for IL-1ra-R polypeptides. However, the IL-1ra-R polypeptide

derivatives disclosed herein may have additional activities, enhanced or reduced biological activity, or other characteristics, such as increased or decreased half-life, as compared to the non-derivatized molecules.

5    Genetically Engineered Non-Human Animals

          Additionally included within the scope of the present invention are non-human animals such as mice, rats, or other rodents; rabbits, goats, sheep, or other farm animals, in which the genes encoding native IL-1ra-R polypeptide have been disrupted (*i.e.*, "knocked out") such that the level of expression of IL-1ra-R  
10   polypeptide is significantly decreased or completely abolished. Such animals may be prepared using techniques and methods such as those described in U.S. Patent No. 5,557,032.

          The present invention further includes non-human animals such as mice, rats, or other rodents; rabbits, goats, sheep, or other farm animals, in which either  
15   the native form of an IL-1ra-R gene for that animal or a heterologous IL-1ra-R gene is over-expressed by the animal, thereby creating a "transgenic" animal. Such transgenic animals may be prepared using well known methods such as those described in U.S. Patent No 5,489,743 and PCT Pub. No. WO 94/28122.

          The present invention further includes non-human animals in which the  
20   promoter for one or more of the IL-1ra-R polypeptides of the present invention is either activated or inactivated (*e.g.*, by using homologous recombination methods) to alter the level of expression of one or more of the native IL-1ra-R polypeptides.

          These non-human animals may be used for drug candidate screening. In such screening, the impact of a drug candidate on the animal may be measured.  
25   For example, drug candidates may decrease or increase the expression of the IL-1ra-R gene. In certain embodiments, the amount of IL-1ra-R polypeptide that is produced may be measured after the exposure of the animal to the drug candidate. Additionally, in certain embodiments, one may detect the actual impact of the drug candidate on the animal. For example, over-expression of a particular gene  
30   may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease expression of the gene

or its ability to prevent or inhibit a pathological condition. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a  
5 metabolic product or its ability to prevent or inhibit a pathological condition.

#### Assaying for Other Modulators of IL-1ra-R Polypeptide Activity

In some situations, it may be desirable to identify molecules that are modulators, *i.e.*, agonists or antagonists, of the activity of IL-1ra-R polypeptide.  
10 Natural or synthetic molecules that modulate IL-1ra-R polypeptide may be identified using one or more screening assays, such as those described herein. Such molecules may be administered either in an *ex vivo* manner or in an *in vivo* manner by injection, or by oral delivery, implantation device, or the like.

"Test molecule" refers to a molecule that is under evaluation for the ability  
15 to modulate (*i.e.*, increase or decrease) the activity of an IL-1ra-R polypeptide. Most commonly, a test molecule will interact directly with an IL-1ra-R polypeptide. However, it is also contemplated that a test molecule may also modulate IL-1ra-R polypeptide activity indirectly, such as by affecting IL-1ra-R gene expression, or by binding to an IL-1ra-R polypeptide binding partner (*e.g.*,  
20 receptor or ligand). In one embodiment, a test molecule will bind to an IL-1ra-R polypeptide with an affinity constant of at least about  $10^{-6}$  M, preferably about  $10^{-8}$  M, more preferably about  $10^{-9}$  M, and even more preferably about  $10^{-10}$  M.

Methods for identifying compounds that interact with IL-1ra-R polypeptides are encompassed by the present invention. In certain embodiments,  
25 an IL-1ra-R polypeptide is incubated with a test molecule under conditions that permit the interaction of the test molecule with an IL-1ra-R polypeptide, and the extent of the interaction is measured. The test molecule can be screened in a substantially purified form or in a crude mixture.

In certain embodiments, an IL-1ra-R polypeptide agonist or antagonist  
30 may be a protein, peptide, carbohydrate, lipid, or small molecular weight molecule that interacts with IL-1ra-R polypeptide to regulate its activity.



Molecules which regulate IL-1ra-R polypeptide expression include nucleic acids which are complementary to nucleic acids encoding an IL-1ra-R polypeptide, or are complementary to nucleic acids sequences which direct or control the expression of IL-1ra-R polypeptide, and which act as anti-sense regulators of expression.

Once a test molecule has been identified as interacting with an IL-1ra-R polypeptide, the molecule may be further evaluated for its ability to increase or decrease IL-1ra-R polypeptide activity. The measurement of the interaction of a test molecule with IL-1ra-R polypeptide may be carried out in several formats, including cell-based binding assays, membrane binding assays, solution-phase assays, and immunoassays. In general, a test molecule is incubated with an IL-1ra-R polypeptide for a specified period of time, and IL-1ra-R polypeptide activity is determined by one or more assays for measuring biological activity.

The interaction of test molecules with IL-1ra-R polypeptides may also be assayed directly using polyclonal or monoclonal antibodies in an immunoassay. Alternatively, modified forms of IL-1ra-R polypeptides containing epitope tags as described herein may be used in solution and immunoassays.

In the event that IL-1ra-R polypeptides display biological activity through an interaction with a binding partner (e.g., a receptor or a ligand), a variety of *in vitro* assays may be used to measure the binding of an IL-1ra-R polypeptide to the corresponding binding partner (such as a selective binding agent, receptor, or ligand). These assays may be used to screen test molecules for their ability to increase or decrease the rate and/or the extent of binding of an IL-1ra-R polypeptide to its binding partner. In one assay, an IL-1ra-R polypeptide is immobilized in the wells of a microtiter plate. Radiolabeled IL-1ra-R polypeptide binding partner (for example, iodinated IL-1ra-R polypeptide binding partner) and a test molecule can then be added either one at a time (in either order) or simultaneously to the wells. After incubation, the wells can be washed and counted for radioactivity, using a scintillation counter, to determine the extent to which the binding partner bound to the IL-1ra-R polypeptide. Typically, a molecule will be tested over a range of concentrations, and a series of control

wells lacking one or more elements of the test assays can be used for accuracy in the evaluation of the results. An alternative to this method involves reversing the "positions" of the proteins, *i.e.*, immobilizing IL-1ra-R polypeptide binding partner to the microtiter plate wells, incubating with the test molecule and radiolabeled IL-1ra-R polypeptide, and determining the extent of IL-1ra-R polypeptide binding. *See, e.g., Current Protocols in Molecular Biology*, chap. 18 (Ausubel *et al.*, eds., Green Publishers Inc. and Wiley and Sons 1995).

As an alternative to radiolabeling, an IL-1ra-R polypeptide or its binding partner may be conjugated to biotin, and the presence of biotinylated protein can then be detected using streptavidin linked to an enzyme, such as horse radish peroxidase (HRP) or alkaline phosphatase (AP), which can be detected colorimetrically, or by fluorescent tagging of streptavidin. An antibody directed to an IL-1ra-R polypeptide or to an IL-1ra-R polypeptide binding partner, and which is conjugated to biotin, may also be used for purposes of detection following incubation of the complex with enzyme-linked streptavidin linked to AP or HRP.

A IL-1ra-R polypeptide or an IL-1ra-R polypeptide binding partner can also be immobilized by attachment to agarose beads, acrylic beads, or other types of such inert solid phase substrates. The substrate-protein complex can be placed in a solution containing the complementary protein and the test compound. After incubation, the beads can be precipitated by centrifugation, and the amount of binding between an IL-1ra-R polypeptide and its binding partner can be assessed using the methods described herein. Alternatively, the substrate-protein complex can be immobilized in a column with the test molecule and complementary protein passing through the column. The formation of a complex between an IL-1ra-R polypeptide and its binding partner can then be assessed using any of the techniques described herein (*e.g.*, radiolabelling or antibody binding).

Another *in vitro* assay that is useful for identifying a test molecule which increases or decreases the formation of a complex between an IL-1ra-R polypeptide binding protein and an IL-1ra-R polypeptide binding partner is a surface plasmon resonance detector system such as the BIAcore assay system

(Pharmacia, Piscataway, NJ). The BIAcore system is utilized as specified by the manufacturer. This assay essentially involves the covalent binding of either IL-1ra-R polypeptide or an IL-1ra-R polypeptide binding partner to a dextran-coated sensor chip that is located in a detector. The test compound and the other  
5 complementary protein can then be injected, either simultaneously or sequentially, into the chamber containing the sensor chip. The amount of complementary protein that binds can be assessed based on the change in molecular mass that is physically associated with the dextran-coated side of the sensor chip, with the change in molecular mass being measured by the detector system.

10 In some cases, it may be desirable to evaluate two or more test compounds together for their ability to increase or decrease the formation of a complex between an IL-1ra-R polypeptide and an IL-1ra-R polypeptide binding partner. In these cases, the assays set forth herein can be readily modified by adding such additional test compound(s) either simultaneously with, or subsequent to, the first  
15 test compound. The remainder of the steps in the assay are as set forth herein.

*In vitro* assays such as those described herein may be used advantageously to screen large numbers of compounds for an effect on the formation of a complex between an IL-1ra-R polypeptide and IL-1ra-R polypeptide binding partner. The assays may be automated to screen compounds generated in phage display,  
20 synthetic peptide, and chemical synthesis libraries.

Compounds which increase or decrease the formation of a complex between an IL-1ra-R polypeptide and an IL-1ra-R polypeptide binding partner may also be screened in cell culture using cells and cell lines expressing either IL-1ra-R polypeptide or IL-1ra-R polypeptide binding partner. Cells and cell lines  
25 may be obtained from any mammal, but preferably will be from human or other primate, canine, or rodent sources. The binding of an IL-1ra-R polypeptide to cells expressing IL-1ra-R polypeptide binding partner at the surface is evaluated in the presence or absence of test molecules, and the extent of binding may be determined by, for example, flow cytometry using a biotinylated antibody to an  
30 IL-1ra-R polypeptide binding partner. Cell culture assays can be used advantageously to further evaluate compounds that score positive in protein

binding assays described herein.

Cell cultures can also be used to screen the impact of a drug candidate. For example, drug candidates may decrease or increase the expression of the IL-1ra-R gene. In certain embodiments, the amount of IL-1ra-R polypeptide or an  
5 IL-1ra-R polypeptide fragment that is produced may be measured after exposure of the cell culture to the drug candidate. In certain embodiments, one may detect the actual impact of the drug candidate on the cell culture. For example, the over-expression of a particular gene may have a particular impact on the cell culture. In such cases, one may test a drug candidate's ability to increase or decrease the  
10 expression of the gene or its ability to prevent or inhibit a particular impact on the cell culture. In other examples, the production of a particular metabolic product such as a fragment of a polypeptide, may result in, or be associated with, a disease or pathological condition. In such cases, one may test a drug candidate's ability to decrease the production of such a metabolic product in a cell culture.

15

#### Internalizing Proteins

The *tat* protein sequence (from HIV) can be used to internalize proteins into a cell. See, e.g., Falwell *et al.*, 1994, *Proc. Natl. Acad. Sci. U.S.A.* 91:664-68. For example, an 11 amino acid sequence (Y-G-R-K-K-R-R-Q-R-R-R; SEQ ID  
20 NO: 18) of the HIV *tat* protein (termed the "protein transduction domain," or TAT PDT) has been described as mediating delivery across the cytoplasmic membrane and the nuclear membrane of a cell. See Schwarze *et al.*, 1999, *Science* 285:1569-72; and Nagahara *et al.*, 1998, *Nat. Med.* 4:1449-52. In these procedures, FITC-constructs (FITC-labeled G-G-G-G-Y-G-R-K-K-R-R-Q-R-R-R; SEQ ID NO: 19),  
25 which penetrate tissues following intraperitoneal administration, are prepared, and the binding of such constructs to cells is detected by fluorescence-activated cell sorting (FACS) analysis. Cells treated with a *tat*- $\beta$ -gal fusion protein will demonstrate  $\beta$ -gal activity. Following injection, expression of such a construct can be detected in a number of tissues, including liver, kidney, lung, heart, and  
30 brain tissue. It is believed that such constructs undergo some degree of unfolding in order to enter the cell, and as such, may require a refolding following entry into

the cell.

It will thus be appreciated that the *tat* protein sequence may be used to internalize a desired polypeptide into a cell. For example, using the *tat* protein sequence, an IL-1ra-R antagonist (such as an anti-IL-1ra-R selective binding agent, small molecule, soluble receptor, or antisense oligonucleotide) can be administered intracellularly to inhibit the activity of an IL-1ra-R molecule. As used herein, the term "IL-1ra-R molecule" refers to both IL-1ra-R nucleic acid molecules and IL-1ra-R polypeptides as defined herein. Where desired, the IL-1ra-R protein itself may also be internally administered to a cell using these procedures. *See also*, Straus, 1999, *Science* 285:1466-67.

#### Cell Source Identification Using IL-1ra-R Polypeptide

In accordance with certain embodiments of the invention, it may be useful to be able to determine the source of a certain cell type associated with an IL-1ra-R polypeptide. For example, it may be useful to determine the origin of a disease or pathological condition as an aid in selecting an appropriate therapy. In certain embodiments, nucleic acids encoding an IL-1ra-R polypeptide can be used as a probe to identify cells described herein by screening the nucleic acids of the cells with such a probe. In other embodiments, one may use anti-IL-1ra-R polypeptide antibodies to test for the presence of IL-1ra-R polypeptide in cells, and thus, determine if such cells are of the types described herein.

#### IL-1ra-R Polypeptide Compositions and Administration

Therapeutic compositions are within the scope of the present invention. Such IL-1RA-R polypeptide pharmaceutical compositions may comprise a therapeutically effective amount of an IL-1ra-R polypeptide or an IL-1ra-R nucleic acid molecule in admixture with a pharmaceutically or physiologically acceptable formulation agent selected for suitability with the mode of administration. Pharmaceutical compositions may comprise a therapeutically effective amount of one or more IL-1ra-R polypeptide selective binding agents in admixture with a pharmaceutically or physiologically acceptable formulation

agent selected for suitability with the mode of administration.

Acceptable formulation materials preferably are nontoxic to recipients at the dosages and concentrations employed.

The pharmaceutical composition may contain formulation materials for  
5 modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine, or lysine), antimicrobials, antioxidants (such as ascorbic  
10 acid, sodium sulfite, or sodium hydrogen-sulfite), buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, or other organic acids), bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)), complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin, or hydroxypropyl-beta-cyclodextrin),  
15 fillers, monosaccharides, disaccharides, and other carbohydrates (such as glucose, mannose, or dextrans), proteins (such as serum albumin, gelatin, or immunoglobulins), coloring, flavoring and diluting agents, emulsifying agents, hydrophilic polymers (such as polyvinylpyrrolidone), low molecular weight polypeptides, salt-forming counterions (such as sodium), preservatives (such as  
20 benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, or hydrogen peroxide), solvents (such as glycerin, propylene glycol, or polyethylene glycol), sugar alcohols (such as mannitol or sorbitol), suspending agents, surfactants or wetting agents (such as pluronics; PEG; sorbitan esters; polysorbates such as polysorbate  
25 20 or polysorbate 80; triton; tromethamine; lecithin; cholesterol or tyloxapal), stability enhancing agents (such as sucrose or sorbitol), tonicity enhancing agents (such as alkali metal halides – preferably sodium or potassium chloride – or mannitol sorbitol), delivery vehicles, diluents, excipients and/or pharmaceutical adjuvants. *See Remington's Pharmaceutical Sciences* (18th Ed., A.R. Gennaro,  
30 ed., Mack Publishing Company 1990.

The optimal pharmaceutical composition will be determined by a skilled

artisan depending upon, for example, the intended route of administration, delivery format, and desired dosage. See, e.g., *Remington's Pharmaceutical Sciences, supra*. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the IL-1ra-R molecule.

5           The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier for injection may be water, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline  
10 mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute. In one embodiment of the present invention, IL-1ra-R polypeptide compositions may be prepared for storage by mixing the selected composition  
15 having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences, supra*) in the form of a lyophilized cake or an aqueous solution. Further, the IL-1ra-R polypeptide product may be formulated as a lyophilizate using appropriate excipients such as sucrose.

          The IL-1ra-R polypeptide pharmaceutical compositions can be selected for  
20 parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

          The formulation components are present in concentrations that are  
25 acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

          When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free,  
30 parenterally acceptable, aqueous solution comprising the desired IL-1ra-R molecule in a pharmaceutically acceptable vehicle. A particularly suitable vehicle

for parenteral injection is sterile distilled water in which an IL-1ra-R molecule is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds  
5 (such as polylactic acid or polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered via a depot injection. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery  
10 devices.

In one embodiment, a pharmaceutical composition may be formulated for inhalation. For example, IL-1ra-R polypeptide may be formulated as a dry powder for inhalation. IL-1ra-R polypeptide or nucleic acid molecule inhalation solutions may also be formulated with a propellant for aerosol delivery. In yet  
15 another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT Pub. No. WO 94/20069, which describes the pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, IL-1ra-R polypeptides that  
20 are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be  
25 included to facilitate absorption of the IL-1ra-R polypeptide. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Another pharmaceutical composition may involve an effective quantity of IL-1ra-R polypeptides in a mixture with non-toxic excipients that are suitable for  
30 the manufacture of tablets. By dissolving the tablets in sterile water, or another appropriate vehicle, solutions can be prepared in unit-dose form. Suitable



excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

5 Additional IL-1ra-R polypeptide pharmaceutical compositions will be evident to those skilled in the art, including formulations involving IL-1ra-R polypeptides in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot  
10 injections, are also known to those skilled in the art. *See, e.g.*, PCT/US93/00829, which describes the controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions.

Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, *e.g.* films, or  
15 microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. Patent No. 3,773,919 and European Patent No. 058481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, 1983, *Biopolymers* 22:547-56), poly(2-hydroxyethyl-methacrylate) (Langer *et al.*, 1981, *J. Biomed. Mater. Res.* 15:167-277 and Langer, 1982, *Chem. Tech.* 12:98-  
20 105), ethylene vinyl acetate (Langer *et al., supra*) or poly-D(-)-3-hydroxybutyric acid (European Patent No. 133988). Sustained-release compositions may also include liposomes, which can be prepared by any of several methods known in the art. *See, e.g.*, Eppstein *et al.*, 1985, *Proc. Natl. Acad. Sci. USA* 82:3688-92; and European Patent Nos. 036676, 088046, and 143949.

25 The IL-1ra-R pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method may be conducted either prior to, or following, lyophilization and reconstitution. The composition for parenteral administration  
30 may be stored in lyophilized form or in a solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for

example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a  
5 ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a  
10 first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

The effective amount of an IL-1ra-R pharmaceutical composition to be  
15 employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the IL-1ra-R molecule is being used, the route of administration, and the size (body weight, body surface, or organ size) and  
20 condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1  $\mu\text{g/kg}$  to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1  $\mu\text{g/kg}$  up to about 100 mg/kg; or 1  
25  $\mu\text{g/kg}$  up to about 100 mg/kg; or 5  $\mu\text{g/kg}$  up to about 100 mg/kg.

The frequency of dosing will depend upon the pharmacokinetic parameters of the IL-1ra-R molecule in the formulation being used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, as two or  
30 more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via an implantation device or

catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

5           The route of administration of the pharmaceutical composition is in accord with known methods, *e.g.*, orally; through injection by intravenous, intraperitoneal, intracerebral (intraparenchymal), intracerebroventricular, intramuscular, intraocular, intraarterial, intraportal, or intralesional routes; by sustained release systems; or by implantation devices. Where desired, the  
10       compositions may be administered by bolus injection or continuously by infusion, or by implantation device.

          Alternatively or additionally, the composition may be administered locally via implantation of a membrane, sponge, or other appropriate material onto which the desired molecule has been absorbed or encapsulated. Where an implantation  
15       device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, timed-release bolus, or continuous administration.

          In some cases, it may be desirable to use IL-1ra-R polypeptide pharmaceutical compositions in an *ex vivo* manner. In such instances, cells,  
20       tissues, or organs that have been removed from the patient are exposed to IL-1ra-R polypeptide pharmaceutical compositions after which the cells, tissues, or organs are subsequently implanted back into the patient.

          In other cases, an IL-1ra-R polypeptide can be delivered by implanting certain cells that have been genetically engineered, using methods such as those  
25       described herein, to express and secrete the IL-1ra-R polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible,  
30       semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune

system or by other detrimental factors from the surrounding tissues.

As discussed herein, it may be desirable to treat isolated cell populations (such as stem cells, lymphocytes, red blood cells, chondrocytes, neurons, and the like) with one or more IL-1ra-R polypeptides. This can be accomplished by exposing the isolated cells to the polypeptide directly, where it is in a form that is permeable to the cell membrane.

Additional embodiments of the present invention relate to cells and methods (e.g., homologous recombination and/or other recombinant production methods) for both the *in vitro* production of therapeutic polypeptides and for the production and delivery of therapeutic polypeptides by gene therapy or cell therapy. Homologous and other recombination methods may be used to modify a cell that contains a normally transcriptionally-silent IL-1ra-R gene, or an under-expressed gene, and thereby produce a cell which expresses therapeutically efficacious amounts of IL-1ra-R polypeptides.

Homologous recombination is a technique originally developed for targeting genes to induce or correct mutations in transcriptionally active genes. Kucherlapati, 1989, *Prog. in Nucl. Acid Res. & Mol. Biol.* 36:301. The basic technique was developed as a method for introducing specific mutations into specific regions of the mammalian genome (Thomas *et al.*, 1986, *Cell* 44:419-28; Thomas and Capecchi, 1987, *Cell* 51:503-12; Doetschman *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:8583-87) or to correct specific mutations within defective genes (Doetschman *et al.*, 1987, *Nature* 330:576-78). Exemplary homologous recombination techniques are described in U.S. Patent No. 5,272,071; European Patent Nos. 9193051 and 505500; PCT/US90/07642, and PCT Pub No. WO 91/09955).

Through homologous recombination, the DNA sequence to be inserted into the genome can be directed to a specific region of the gene of interest by attaching it to targeting DNA. The targeting DNA is a nucleotide sequence that is complementary (homologous) to a region of the genomic DNA. Small pieces of targeting DNA that are complementary to a specific region of the genome are put in contact with the parental strand during the DNA replication process. It is a

general property of DNA that has been inserted into a cell to hybridize, and therefore, recombine with other pieces of endogenous DNA through shared homologous regions. If this complementary strand is attached to an oligonucleotide that contains a mutation or a different sequence or an additional  
5 nucleotide, it too is incorporated into the newly synthesized strand as a result of the recombination. As a result of the proofreading function, it is possible for the new sequence of DNA to serve as the template. Thus, the transferred DNA is incorporated into the genome.

Attached to these pieces of targeting DNA are regions of DNA that may  
10 interact with or control the expression of an IL-1ra-R polypeptide, *e.g.*, flanking sequences. For example, a promoter/enhancer element, a suppressor, or an exogenous transcription modulatory element is inserted in the genome of the intended host cell in proximity and orientation sufficient to influence the transcription of DNA encoding the desired IL-1ra-R polypeptide. The control  
15 element controls a portion of the DNA present in the host cell genome. Thus, the expression of the desired IL-1ra-R polypeptide may be achieved not by transfection of DNA that encodes the IL-1ra-R gene itself, but rather by the use of targeting DNA (containing regions of homology with the endogenous gene of interest) coupled with DNA regulatory segments that provide the endogenous gene  
20 sequence with recognizable signals for transcription of an IL-1ra-R gene.

In an exemplary method, the expression of a desired targeted gene in a cell (*i.e.*, a desired endogenous cellular gene) is altered via homologous recombination into the cellular genome at a preselected site, by the introduction of DNA which includes at least a regulatory sequence, an exon, and a splice donor site. These  
25 components are introduced into the chromosomal (genomic) DNA in such a manner that this, in effect, results in the production of a new transcription unit (in which the regulatory sequence, the exon, and the splice donor site present in the DNA construct are operatively linked to the endogenous gene). As a result of the introduction of these components into the chromosomal DNA, the expression of  
30 the desired endogenous gene is altered.

Altered gene expression, as described herein, encompasses activating (or

causing to be expressed) a gene which is normally silent (unexpressed) in the cell as obtained, as well as increasing the expression of a gene which is not expressed at physiologically significant levels in the cell as obtained. The embodiments further encompass changing the pattern of regulation or induction such that it is different from the pattern of regulation or induction that occurs in the cell as obtained, and reducing (including eliminating) the expression of a gene which is expressed in the cell as obtained.

One method by which homologous recombination can be used to increase, or cause, IL-1ra-R polypeptide production from a cell's endogenous IL-1ra-R gene involves first using homologous recombination to place a recombination sequence from a site-specific recombination system (e.g., Cre/loxP, FLP/FRT) (Sauer, 1994, *Curr. Opin. Biotechnol.*, 5:521-27; Sauer, 1993, *Methods Enzymol.*, 225:890-900) upstream of (i.e., 5' to) the cell's endogenous genomic IL-1ra-R polypeptide coding region. A plasmid containing a recombination site homologous to the site that was placed just upstream of the genomic IL-1ra-R polypeptide coding region is introduced into the modified cell line along with the appropriate recombinase enzyme. This recombinase causes the plasmid to integrate, via the plasmid's recombination site, into the recombination site located just upstream of the genomic IL-1ra-R polypeptide coding region in the cell line (Baubonis and Sauer, 1993, *Nucleic Acids Res.* 21:2025-29; O'Gorman *et al.*, 1991, *Science* 251:1351-55). Any flanking sequences known to increase transcription (e.g., enhancer/promoter, intron, translational enhancer), if properly positioned in this plasmid, would integrate in such a manner as to create a new or modified transcriptional unit resulting in *de novo* or increased IL-1ra-R polypeptide production from the cell's endogenous IL-1ra-R gene.

A further method to use the cell line in which the site specific recombination sequence had been placed just upstream of the cell's endogenous genomic IL-1ra-R polypeptide coding region is to use homologous recombination to introduce a second recombination site elsewhere in the cell line's genome. The appropriate recombinase enzyme is then introduced into the two-recombination-site cell line, causing a recombination event (deletion, inversion, and

translocation) (Sauer, 1994, *Curr. Opin. Biotechnol.*, 5:521-27; Sauer, 1993, *Methods Enzymol.*, 225:890-900) that would create a new or modified transcriptional unit resulting in *de novo* or increased IL-1ra-R polypeptide production from the cell's endogenous IL-1ra-R gene.

5           An additional approach for increasing, or causing, the expression of IL-1ra-R polypeptide from a cell's endogenous IL-1ra-R gene involves increasing, or causing, the expression of a gene or genes (*e.g.*, transcription factors) and/or decreasing the expression of a gene or genes (*e.g.*, transcriptional repressors) in a manner which results in *de novo* or increased IL-1ra-R polypeptide production  
10       from the cell's endogenous IL-1ra-R gene. This method includes the introduction of a non-naturally occurring polypeptide (*e.g.*, a polypeptide comprising a site specific DNA binding domain fused to a transcriptional factor domain) into the cell such that *de novo* or increased IL-1ra-R polypeptide production from the cell's endogenous IL-1ra-R gene results.

15           The present invention further relates to DNA constructs useful in the method of altering expression of a target gene. In certain embodiments, the exemplary DNA constructs comprise: (a) one or more targeting sequences, (b) a regulatory sequence, (c) an exon, and (d) an unpaired splice-donor site. The targeting sequence in the DNA construct directs the integration of elements (a) -  
20       (d) into a target gene in a cell such that the elements (b) - (d) are operatively linked to sequences of the endogenous target gene. In another embodiment, the DNA constructs comprise: (a) one or more targeting sequences, (b) a regulatory sequence, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site, wherein the targeting sequence directs the integration of elements (a)  
25       - (f) such that the elements of (b) - (f) are operatively linked to the endogenous gene. The targeting sequence is homologous to the preselected site in the cellular chromosomal DNA with which homologous recombination is to occur. In the construct, the exon is generally 3' of the regulatory sequence and the splice-donor site is 3' of the exon.

30           If the sequence of a particular gene is known, such as the nucleic acid sequence of IL-1ra-R polypeptide presented herein, a piece of DNA that is

complementary to a selected region of the gene can be synthesized or otherwise obtained, such as by appropriate restriction of the native DNA at specific recognition sites bounding the region of interest. This piece serves as a targeting sequence upon insertion into the cell and will hybridize to its homologous region within the genome. If this hybridization occurs during DNA replication, this piece of DNA, and any additional sequence attached thereto, will act as an Okazaki fragment and will be incorporated into the newly synthesized daughter strand of DNA. The present invention, therefore, includes nucleotides encoding an IL-1ra-R polypeptide, which nucleotides may be used as targeting sequences.

IL-1ra-R polypeptide cell therapy, *e.g.*, the implantation of cells producing IL-1ra-R polypeptides, is also contemplated. This embodiment involves implanting cells capable of synthesizing and secreting a biologically active form of IL-1ra-R polypeptide. Such IL-1ra-R polypeptide-producing cells can be cells that are natural producers of IL-1ra-R polypeptides or may be recombinant cells whose ability to produce IL-1ra-R polypeptides has been augmented by transformation with a gene encoding the desired IL-1ra-R polypeptide or with a gene augmenting the expression of IL-1ra-R polypeptide. Such a modification may be accomplished by means of a vector suitable for delivering the gene as well as promoting its expression and secretion. In order to minimize a potential immunological reaction in patients being administered an IL-1ra-R polypeptide, as may occur with the administration of a polypeptide of a foreign species, it is preferred that the natural cells producing IL-1ra-R polypeptide be of human origin and produce human IL-1ra-R polypeptide. Likewise, it is preferred that the recombinant cells producing IL-1ra-R polypeptide be transformed with an expression vector containing a gene encoding a human IL-1ra-R polypeptide.

Implanted cells may be encapsulated to avoid the infiltration of surrounding tissue. Human or non-human animal cells may be implanted in patients in biocompatible, semipermeable polymeric enclosures or membranes that allow the release of IL-1ra-R polypeptide, but that prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissue. Alternatively, the patient's own cells, transformed to produce



IL-1ra-R polypeptides *ex vivo*, may be implanted directly into the patient without such encapsulation.

Techniques for the encapsulation of living cells are known in the art, and the preparation of the encapsulated cells and their implantation in patients may be routinely accomplished. For example, Baetge *et al.* (PCT Pub. No. WO 95/05452 and PCT/US94/09299) describe membrane capsules containing genetically engineered cells for the effective delivery of biologically active molecules. The capsules are biocompatible and are easily retrievable. The capsules encapsulate cells transfected with recombinant DNA molecules comprising DNA sequences coding for biologically active molecules operatively linked to promoters that are not subject to down-regulation *in vivo* upon implantation into a mammalian host. The devices provide for the delivery of the molecules from living cells to specific sites within a recipient. In addition, see U.S. Patent Nos. 4,892,538; 5,011,472; and 5,106,627. A system for encapsulating living cells is described in PCT Pub. No. WO 91/10425 (Aebischer *et al.*). See also, PCT Pub. No. WO 91/10470 (Aebischer *et al.*); Winn *et al.*, 1991, *Exper. Neurol.* 113:322-29; Aebischer *et al.*, 1991, *Exper. Neurol.* 111:269-75; and Tresco *et al.*, 1992, *ASAIO* 38:17-23.

*In vivo* and *in vitro* gene therapy delivery of IL-1ra-R polypeptides is also envisioned. One example of a gene therapy technique is to use the IL-1ra-R gene (either genomic DNA, cDNA, and/or synthetic DNA) encoding an IL-1ra-R polypeptide which may be operably linked to a constitutive or inducible promoter to form a "gene therapy DNA construct." The promoter may be homologous or heterologous to the endogenous IL-1ra-R gene, provided that it is active in the cell or tissue type into which the construct will be inserted. Other components of the gene therapy DNA construct may optionally include DNA molecules designed for site-specific integration (*e.g.*, endogenous sequences useful for homologous recombination), tissue-specific promoters, enhancers or silencers, DNA molecules capable of providing a selective advantage over the parent cell, DNA molecules useful as labels to identify transformed cells, negative selection systems, cell specific binding agents (as, for example, for cell targeting), cell-specific internalization factors, transcription factors enhancing expression from a vector,

and factors enabling vector production.

A gene therapy DNA construct can then be introduced into cells (either *ex vivo* or *in vivo*) using viral or non-viral vectors. One means for introducing the gene therapy DNA construct is by means of viral vectors as described herein.

- 5 Certain vectors, such as retroviral vectors, will deliver the DNA construct to the chromosomal DNA of the cells, and the gene can integrate into the chromosomal DNA. Other vectors will function as episomes, and the gene therapy DNA construct will remain in the cytoplasm.

- 10 In yet other embodiments, regulatory elements can be included for the controlled expression of the IL-1ra-R gene in the target cell. Such elements are turned on in response to an appropriate effector. In this way, a therapeutic polypeptide can be expressed when desired. One conventional control means involves the use of small molecule dimerizers or rapalogs to dimerize chimeric proteins which contain a small molecule-binding domain and a domain capable of  
15 initiating a biological process, such as a DNA-binding protein or transcriptional activation protein (*see* PCT Pub. Nos. WO 96/41865, WO 97/31898, and WO 97/31899). The dimerization of the proteins can be used to initiate transcription of the transgene.

- 20 An alternative regulation technology uses a method of storing proteins expressed from the gene of interest inside the cell as an aggregate or cluster. The gene of interest is expressed as a fusion protein that includes a conditional aggregation domain that results in the retention of the aggregated protein in the endoplasmic reticulum. The stored proteins are stable and inactive inside the cell. The proteins can be released, however, by administering a drug (*e.g.*, small  
25 molecule ligand) that removes the conditional aggregation domain and thereby specifically breaks apart the aggregates or clusters so that the proteins may be secreted from the cell. *See* Aridor *et al.*, 2000, *Science* 287:816-17 and Rivera *et al.*, 2000, *Science* 287:826-30.

- 30 Other suitable control means or gene switches include, but are not limited to, the systems described herein. Mifepristone (RU486) is used as a progesterone antagonist. The binding of a modified progesterone receptor ligand-binding

domain to the progesterone antagonist activates transcription by forming a dimer of two transcription factors that then pass into the nucleus to bind DNA. The ligand-binding domain is modified to eliminate the ability of the receptor to bind to the natural ligand. The modified steroid hormone receptor system is further  
5 described in U.S. Patent No. 5,364,791 and PCT Pub. Nos. WO 96/40911 and WO 97/10337.

Yet another control system uses ecdysone (a fruit fly steroid hormone) which binds to and activates an ecdysone receptor (cytoplasmic receptor). The receptor then translocates to the nucleus to bind a specific DNA response element  
10 (promoter from ecdysone-responsive gene). The ecdysone receptor includes a transactivation domain, DNA-binding domain, and ligand-binding domain to initiate transcription. The ecdysone system is further described in U.S. Patent No. 5,514,578 and PCT Pub. Nos. WO 97/38117, WO 96/37609, and WO 93/03162.

Another control means uses a positive tetracycline-controllable  
15 transactivator. This system involves a mutated tet repressor protein DNA-binding domain (mutated tet R-4 amino acid changes which resulted in a reverse tetracycline-regulated transactivator protein, *i.e.*, it binds to a tet operator in the presence of tetracycline) linked to a polypeptide which activates transcription. Such systems are described in U.S. Patent Nos. 5,464,758, 5,650,298, and  
20 5,654,168.

Additional expression control systems and nucleic acid constructs are described in U.S. Patent Nos. 5,741,679 and 5,834,186, to Innovir Laboratories Inc.

*In vivo* gene therapy may be accomplished by introducing the gene  
25 encoding IL-1ra-R polypeptide into cells via local injection of an IL-1ra-R nucleic acid molecule or by other appropriate viral or non-viral delivery vectors. Hefti, 1994, *Neurobiology* 25:1418-35. For example, a nucleic acid molecule encoding an IL-1ra-R polypeptide may be contained in an adeno-associated virus (AAV) vector for delivery to the targeted cells (*see, e.g.*, Johnson, PCT Pub. No. WO  
30 95/34670; PCT App. No. PCT/US95/07178). The recombinant AAV genome typically contains AAV inverted terminal repeats flanking a DNA sequence

encoding an IL-1ra-R polypeptide operably linked to functional promoter and polyadenylation sequences.

Alternative suitable viral vectors include, but are not limited to, retrovirus, adenovirus, herpes simplex virus, lentivirus, hepatitis virus, parvovirus, papovavirus, poxvirus, alphavirus, coronavirus, rhabdovirus, paramyxovirus, and papilloma virus vectors. U.S. Patent No. 5,672,344 describes an *in vivo* viral-mediated gene transfer system involving a recombinant neurotrophic HSV-1 vector. U.S. Patent No. 5,399,346 provides examples of a process for providing a patient with a therapeutic protein by the delivery of human cells which have been treated *in vitro* to insert a DNA segment encoding a therapeutic protein. Additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent Nos. 5,631,236 (involving adenoviral vectors), 5,672,510 (involving retroviral vectors), 5,635,399 (involving retroviral vectors expressing cytokines).

Nonviral delivery methods include, but are not limited to, liposome-mediated transfer, naked DNA delivery (direct injection), receptor-mediated transfer (ligand-DNA complex), electroporation, calcium phosphate precipitation, and microparticle bombardment (*e.g.*, gene gun). Gene therapy materials and methods may also include inducible promoters, tissue-specific enhancer-promoters, DNA sequences designed for site-specific integration, DNA sequences capable of providing a selective advantage over the parent cell, labels to identify transformed cells, negative selection systems and expression control systems (safety measures), cell-specific binding agents (for cell targeting), cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as methods of vector manufacture. Such additional methods and materials for the practice of gene therapy techniques are described in U.S. Patent Nos. 4,970,154 (involving electroporation techniques), 5,679,559 (describing a lipoprotein-containing system for gene delivery), 5,676,954 (involving liposome carriers), 5,593,875 (describing methods for calcium phosphate transfection), and 4,945,050 (describing a process wherein biologically active particles are propelled at cells at a speed whereby the particles penetrate the surface of the cells and

become incorporated into the interior of the cells), and PCT Pub. No. WO 96/40958 (involving nuclear ligands).

It is also contemplated that IL-1ra-R gene therapy or cell therapy can further include the delivery of one or more additional polypeptide(s) in the same or a different cell(s). Such cells may be separately introduced into the patient, or  
5 the cells may be contained in a single implantable device, such as the encapsulating membrane described above, or the cells may be separately modified by means of viral vectors.

A means to increase endogenous IL-1ra-R polypeptide expression in a cell  
10 via gene therapy is to insert one or more enhancer elements into the IL-1ra-R polypeptide promoter, where the enhancer elements can serve to increase transcriptional activity of the IL-1ra-R gene. The enhancer elements used will be selected based on the tissue in which one desires to activate the gene – enhancer elements known to confer promoter activation in that tissue will be selected. For  
15 example, if a gene encoding an IL-1ra-R polypeptide is to be “turned on” in T-cells, the *lck* promoter enhancer element may be used. Here, the functional portion of the transcriptional element to be added may be inserted into a fragment of DNA containing the IL-1ra-R polypeptide promoter (and optionally, inserted into a vector and/or 5’ and/or 3’ flanking sequences) using standard cloning  
20 techniques. This construct, known as a “homologous recombination construct,” can then be introduced into the desired cells either *ex vivo* or *in vivo*.

Gene therapy also can be used to decrease IL-1ra-R polypeptide expression by modifying the nucleotide sequence of the endogenous promoter. Such modification is typically accomplished via homologous recombination  
25 methods. For example, a DNA molecule containing all or a portion of the promoter of the IL-1ra-R gene selected for inactivation can be engineered to remove and/or replace pieces of the promoter that regulate transcription. For example, the TATA box and/or the binding site of a transcriptional activator of the promoter may be deleted using standard molecular biology techniques; such  
30 deletion can inhibit promoter activity thereby repressing the transcription of the corresponding IL-1ra-R gene. The deletion of the TATA box or the transcription

activator binding site in the promoter may be accomplished by generating a DNA construct comprising all or the relevant portion of the IL-1ra-R polypeptide promoter (from the same or a related species as the IL-1ra-R gene to be regulated) in which one or more of the TATA box and/or transcriptional activator binding site nucleotides are mutated via substitution, deletion and/or insertion of one or more nucleotides. As a result, the TATA box and/or activator binding site has decreased activity or is rendered completely inactive. This construct, which also will typically contain at least about 500 bases of DNA that correspond to the native (endogenous) 5' and 3' DNA sequences adjacent to the promoter segment that has been modified, may be introduced into the appropriate cells (either *ex vivo* or *in vivo*) either directly or via a viral vector as described herein. Typically, the integration of the construct into the genomic DNA of the cells will be via homologous recombination, where the 5' and 3' DNA sequences in the promoter construct can serve to help integrate the modified promoter region via hybridization to the endogenous chromosomal DNA.

#### Therapeutic Uses

IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists thereof can be used to treat, diagnose, ameliorate, or prevent a number of diseases, disorders, or conditions, including those recited herein.

IL-1ra-R polypeptide agonists and antagonists include those molecules which regulate IL-1ra-R polypeptide activity and either increase or decrease at least one activity of the mature form of the IL-1ra-R polypeptide. Agonists or antagonists may be co-factors, such as a protein, peptide, carbohydrate, lipid, or small molecular weight molecule, which interact with IL-1ra-R polypeptide and thereby regulate its activity. Potential polypeptide agonists or antagonists include antibodies that react with either soluble or membrane-bound forms of IL-1ra-R polypeptides that comprise part or all of the extracellular domains of the said proteins. Molecules that regulate IL-1ra-R polypeptide expression typically include nucleic acids encoding IL-1ra-R polypeptide that can act as anti-sense regulators of expression.

For example, the IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can be used to treat, diagnose, ameliorate, or prevent diseases, disorders, or conditions involving immune system dysfunction. Examples of such diseases include, but are not limited to, 5 rheumatoid arthritis, psoriatic arthritis, inflammatory arthritis, osteoarthritis, inflammatory joint disease, autoimmune disease (including autoimmune vasculitis), multiple sclerosis, lupus, diabetes (e.g., insulin diabetes), inflammatory bowel disease, transplant rejection, graft versus host disease, and inflammatory conditions resulting from strain, sprain, cartilage damage, trauma, 10 orthopedic surgery, infection or other disease processes. Other diseases influenced by the dysfunction of the immune system are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or 15 prevent diseases, disorders, or conditions involving infection. Examples of such diseases include, but are not limited to, leprosy, viral infections (such as hepatitis or HIV), bacterial infection (such as *clostridium*-associated illnesses, including *clostridium*-associated diarrhea), pulmonary tuberculosis, acute febrile illness, fever, acute phase response of the liver, septicemia, or septic shock. Other 20 diseases involving infection are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or prevent diseases, disorders, or conditions involving weight disorders. Examples of such diseases include, but are not limited to obesity, anorexia, cachexia 25 (including AIDS-induced cachexia), myopathies (e.g., muscle protein metabolism, such as in sepsis), and hypoglycemia. Other diseases involving weight disorders are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or 30 prevent diseases, disorders, or conditions involving neuronal dysfunction. Examples of such diseases include, but are not limited to, Alzheimer's disease,

Parkinson's disease, neurotoxicity (e.g., as induced by HIV), ALS, brain injury, stress, depression, nociception and other pain (including cancer-related pain), hyperalgesia, epilepsy, learning impairment and memory disorders, sleep disturbance, and peripheral and central neuropathies. Other neurological disorders  
5 are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or prevent diseases, disorders, or conditions involving the lung. Examples of such diseases include, but are not limited to, acute or chronic lung injury (including  
10 interstitial lung disease), acute respiratory disease syndrome, pulmonary hypertension, emphysema, cystic fibrosis, pulmonary fibrosis, and asthma. Other diseases of the lung are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or  
15 prevent diseases, disorders, or conditions involving the skin. Examples of such diseases include, but are not limited to, psoriasis, eczema, and wound healing. Other diseases of the skin are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or  
20 prevent diseases, disorders, or conditions involving the kidney. Examples of such diseases include, but are not limited to, acute and chronic glomerulonephritis. Other diseases of the kidney are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or  
25 prevent diseases, disorders, or conditions involving the bone. Examples of such diseases include, but are not limited to, osteoporosis, osteopetrosis, osteogenesis imperfecta, Paget's disease, periodontal disease, temporal mandibular joint disease, and hypercalcemia. Other diseases of the bone are encompassed within the scope of the invention.

30 The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or



prevent diseases, disorders, or conditions involving the vascular system. Examples of such diseases include, but are not limited to, hemorrhage or stroke, hemorrhagic shock, ischemia (including cardiac ischemia and cerebral ischemia, *e.g.*, brain injury as a result of trauma, epilepsy, hemorrhage or stroke, each of which may lead to neurodegeneration), atherosclerosis, congestive heart failure, 5 restenosis, reperfusion injury, and angiogenesis. Other diseases of the vascular system are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or 10 prevent diseases, disorders, or conditions involving tumor cells. Examples of such diseases include, but are not limited to, lymphomas, bone sarcoma, chronic and acute myelogenous leukemia (CML and AML) and other leukemias, multiple myeloma, lung cancer, breast cancer, tumor metastasis, and side effects from radiation therapy. Other diseases involving tumor cells are encompassed within 15 the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or prevent diseases, disorders, or conditions involving the reproductive system. Examples of such diseases include, but are not limited to, infertility, miscarriage, 20 pre-term labor and delivery, and endometriosis. Other diseases involving the reproductive system are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and antagonists of the invention can also be used to treat, diagnose, ameliorate, or prevent diseases, disorders, or conditions involving the eye. Examples of such 25 diseases include, but are not limited to, inflammatory eye disease (as may be associated with, for example, corneal transplant), retinal degeneration, blindness, macular degeneration, glaucoma, uveitis, and retinal neuropathy. Other diseases of the eye are encompassed within the scope of the invention.

The IL-1ra-R nucleic acid molecules, polypeptides, and agonists and 30 antagonists of the invention can also be used to treat diseases such as acute pancreatitis, chronic fatigue syndrome, fibromyalgia, and Kawasaki's disease

(MLNS).

IL-1 inhibitors include any protein capable of specifically preventing activation of cellular receptors to IL-1, which may result from any number of mechanisms. Such mechanisms include down-regulating IL-1 production, binding  
5 free IL-1, interfering with IL-1 binding to its receptor, interfering with the formation of the IL-1 receptor complex (*i.e.*, association of IL-1 receptor with IL-1 receptor accessory protein), and interfering with the modulation of IL-1 signaling after binding to its receptor. Such interleukin-1 inhibitors include: interleukin-1 receptor antagonists such as IL-1ra-R, as described herein, anti-IL-1  
10 receptor monoclonal antibodies (*e.g.*, European Patent No. 623674), IL-1 binding proteins such as soluble IL-1 receptors (*e.g.*, U.S. Patent Nos. 5,492,888, 5,488,032, 5,464,937, 5,319,071, and 5,180,812), anti-IL-1 monoclonal antibodies (*e.g.*, PCT Pub. Nos. WO 95/01997, WO 94/02627, WO 90/06371; U.S. Patent No. 4,935,343; and European Patent Nos. 364778, 267611 and 220063), IL-1  
15 receptor accessory proteins and antibodies thereto (*e.g.*, PCT Pub. No. WO 96/23067); inhibitors of interleukin-1 $\beta$  converting enzyme (ICE) or caspase I, which can be used to inhibit IL-1 $\beta$  production and secretion, interleukin-1 $\beta$  protease inhibitors, and other compounds and proteins which block *in vivo* synthesis or extracellular release of IL-1.

20 Exemplary IL-1 inhibitors are disclosed in US Patent Nos. 5,747,444, 5,359,032, 5,608,035, 5,843,905, 5,359,032, 5,866,576, 5,869,660, 5,869,315, 5,872,095, 5,955,480; PCT Pub. Nos. WO 98/21957, WO 96/09323, WO 91/17184, WO 96/40907, WO 98/32733, WO 98/42325, WO 98/44940, WO 98/47892, WO 98/56377, WO 99/03837, WO 99/06426, WO 99/06042, WO  
25 91/17249, WO 98/32733, WO 98/17661, WO 97/08174, WO 95/34326, WO 99/36426, and WO 99/36415; European Patent Nos. 534978 and 894795; and French Patent Application FR 2762514.

Interleukin-1 receptor antagonist (IL-1ra) is a human protein that acts as a natural inhibitor of interleukin-1. Preferred receptor antagonists (including IL-1ra  
30 and variants and derivatives thereof), as well as methods of making and using thereof, are described in U.S. Patent No. 5,075,222; PCT Pub. Nos. WO

91/08285, WO 91/17184, WO 92/16221, WO 93/21946, WO 94/06457, WO 94/21275, WO 94/21235, WO 94/20517, WO 96/22793, WO 97/28828, and WO 99/36541; Austrian Patent No. AU 9173636; French Patent No. FR 2706772; and German Patent No. DE 4219626. Such proteins include glycosylated as well as  
5 non-glycosylated IL-1 receptor antagonists.

Specifically, three exemplary forms of IL-1ra and variants thereof are disclosed and described in the 5,075,222 patent. The first of these, called "IL-1i," is characterized as a 22-23 kD molecule on SDS-PAGE with an approximate isoelectric point of 4.8, eluting from a MonoQ FPLC column at around 52 mM  
10 NaCl in Tris buffer, pH 7.6. The second, IL-1ra $\beta$ , is characterized as a 22-23 kD protein, eluting from a MonoQ column at 48 mM NaCl. Both IL-1ra $\alpha$  and IL-1ra $\beta$  are glycosylated. The third, IL-1ra $\chi$ , is characterized as a 20 kD protein, eluting from a MonoQ column at 48 mM NaCl, and is non-glycosylated. U.S. Patent No. 5,075,222 also discloses methods for isolating the genes responsible  
15 for coding the inhibitors, cloning the gene in suitable vectors and cell types, and expressing the gene to produce the inhibitors.

Those skilled in the art will recognize that many combinations of deletions, insertions, and substitutions (individually or collectively "variant(s)" herein) can be made within the amino acid sequences of IL-1ra-R, provided that  
20 the resulting molecule is biologically active (*e.g.*, possesses the ability to affect one or more of the diseases and disorders such as those recited herein.)

As contemplated by the present invention, an IL-1ra-R polypeptide may be administered as an adjunct to other therapy and also with other pharmaceutical compositions suitable for the indication being treated. An IL-1ra-R polypeptide  
25 and any of one or more additional therapies or pharmaceutical formulations may be administered separately, sequentially, or simultaneously.

In a specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pre-treatment, post-treatment, or concurrent treatment) with any of one or more TNF inhibitors for the treatment or prevention  
30 of the diseases and disorders recited herein.

Such TNF inhibitors include compounds and proteins that block *in vivo*

synthesis or extracellular release of TNF. In a specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pre-treatment, post-treatment, or concurrent treatment) with any of one or more of the following TNF inhibitors: TNF binding proteins (soluble TNF receptor type-I and soluble TNF receptor type-II ("sTNFRs"), as defined herein), anti-TNF antibodies, granulocyte colony stimulating factor, thalidomide, BN 50730, tenidap, E 5531, tiapafant PCA 4248, nimesulide, panavir, rolipram, RP 73401, peptide T, MDL 201,449A, (1R,3S)-Cis-1-[9-(2,6-diaminopuriny)]-3-hydroxy-4-cyclopentene hydrochloride, (1R,3R)-trans-1-(9-(2,6-diamino)purine]-3-acetoxycyclopentane, (1R,3R)-trans-1-[9-adenyl]-3-azidocyclopentane hydrochloride and (1R,3R)-trans-1-(6-hydroxy-purin-9-yl)-3-azidocyclopentane. TNF binding proteins are disclosed in the art (U.S. Patent No. 5,136,021; European Patent Nos. 308378, 422339, 393438, 398327, 412486, 418014, 433900, 464533, 512528, 526905, 568928, 417563; PCT Pub. Nos. WO 90/13575, WO 91/03553, WO 92/01002, WO 92/13095, WO 92/16221, WO 93/07863, WO 93/21946, WO 93/19777, WO 94/06476, PCT App. No. PCT/US97/12244; English Patent Nos. GB 2218101 and 2246569; and Japanese Patent App. No. JP 127,800/1991).

For example, European Patent Nos. 393438 and 422339 teach the amino acid and nucleic acid sequences of a soluble TNF receptor type I (also known as "sTNFR-I" or "30kDa TNF inhibitor") and a soluble TNF receptor type II (also known as "sTNFR-II" or "40kDa TNF inhibitor"), collectively termed "sTNFRs," as well as modified forms thereof (*e.g.*, fragments, functional derivatives, and variants). European Patent Nos. 393438 and 422339 also disclose methods for isolating the genes responsible for coding the inhibitors, cloning the gene in suitable vectors and cell types, and expressing the gene to produce the inhibitors. Additionally, polyvalent forms (*i.e.*, molecules comprising more than one active moiety) of sTNFR-I and sTNFR-II have also been disclosed. In one embodiment, the polyvalent form may be constructed by chemically coupling at least one TNF inhibitor and another moiety with any clinically acceptable linker, for example polyethylene glycol (PCT Pub. Nos. WO 92/16221 and WO 95/34326), by a

peptide linker (Neve *et al.*, 1996, *Cytokine*, 8:365-70), by chemically coupling to biotin and then binding to avidin (PCT Pub. No. WO 91/03553) and, finally, by combining chimeric antibody molecules (U.S. Patent No. 5,116,964; PCT Pub. Nos. WO 89/09622 and WO 91/16437; and European Patent No. 315062).

5        Anti-TNF antibodies include MAK 195F Fab antibody (Holler *et al.*, 1993, *1st International Symposium on Cytokines in Bone Marrow Transplantation* 147), CDP 571 anti-TNF monoclonal antibody (Rankin *et al.*, 1995, *Br. J. Rheumatol.*, 34:334-42), BAY X 1351 murine anti-tumor necrosis factor monoclonal antibody (Kieft *et al.*, 1995, *7th European Congress of Clinical Microbiology and Infectious Diseases* 9); CenTNF cA2 anti-TNF monoclonal antibody (Elliott *et al.*,  
10        1994, *Lancet*, 344:1125-27; Elliott *et al.*, 1994, *Lancet*, 344:1105-10).

         In a specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with secreted or soluble human fas antigen or recombinant versions thereof (PCT Pub. No. WO 96/20206; Mountz *et al.*, 1995, *J. Immunol.*,  
15        155:4829-37; and European Patent No. 510691). PCT Pub. No. WO 96/20206 discloses secreted human fas antigen (native and recombinant, including an Ig fusion protein), methods for isolating the genes responsible for coding the soluble recombinant human fas antigen, methods for cloning the gene in suitable vectors and cell types, and methods for expressing the gene to produce the inhibitors.  
20        European Patent No. 510691 teaches nucleic acids coding for human fas antigen, including soluble fas antigen, vectors expressing for said nucleic acids, and transformants transfected with the vector. When administered parenterally, doses of a secreted or soluble fas antigen fusion protein each are generally from about 1  
25        µg/kg to about 100 µg/kg.

         Current treatment of the diseases and disorders recited herein, including acute and chronic inflammation such as rheumatic diseases, commonly includes the use of first line drugs for control of pain and inflammation; these drugs are classified as non-steroidal, anti-inflammatory drugs (NSAIDs). Secondary  
30        treatments include corticosteroids, slow acting antirheumatic drugs (SAARDs), or disease modifying (DM) drugs. Information regarding the following compounds

can be found in *The Merck Manual of Diagnosis and Therapy* (16th ed. 1992) and in *Pharmaprojects* (PJB Publications Ltd).

In a specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide and any of one or more NSAIDs for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation such as rheumatic diseases, and graft versus host disease. NSAIDs owe their anti-inflammatory action, at least in part, to the inhibition of prostaglandin synthesis (Goodman and Gilman, *The Pharmacological Basis of Therapeutics* (7th ed. 1985)). NSAIDs can be characterized into at least nine groups: (1) salicylic acid derivatives, (2) propionic acid derivatives, (3) acetic acid derivatives, (4) fenamic acid derivatives, (5) carboxylic acid derivatives, (6) butyric acid derivatives, (7) oxicams, (8) pyrazoles, and (9) pyrazolones.

In another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more salicylic acid derivatives, prodrug esters, or pharmaceutically acceptable salts thereof. Such salicylic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof comprise: acetaminosalol, aloxiprin, aspirin, benorylate, bromosaligenin, calcium acetylsalicylate, choline magnesium trisalicylate, magnesium salicylate, choline salicylate, diflusal, etersalate, fendosal, gentisic acid, glycol salicylate, imidazole salicylate, lysine acetylsalicylate, mesalamine, morpholine salicylate, 1-naphthyl salicylate, olsalazine, parsalimide, phenyl acetylsalicylate, phenyl salicylate, salacetamide, salicylamide O-acetic acid, salsalate, sodium salicylate and sulfasalazine. Structurally related salicylic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In an additional specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more propionic acid derivatives, prodrug esters, or pharmaceutically acceptable salts thereof. The propionic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof

comprise: alminoprofen, benoxaprofen, bucloxic acid, carprofen, dexindoprofen, fenoprofen, flunoxaprofen, fluprofen, flurbiprofen, furciprofen, ibuprofen, ibuprofen aluminum, ibuprofen, indoprofen, isoprofen, ketoprofen, loxoprofen, mioprofen, naproxen, naproxen sodium, oxaprozin, piketoprofen, pimeprofen, 5 pirprofen, pranoprofen, protizinic acid, pyridoxiprofen, suprofen, tiaprofenic acid and tioxaprofen. Structurally related propionic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In yet another specific embodiment, the present invention is directed to the 10 use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more acetic acid derivatives, prodrug esters, or pharmaceutically acceptable salts thereof. The acetic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof comprise: acemetacin, alclofenac, amfenac, bufexamac, cinmetacin, clopirac, delmetacin, 15 diclofenac potassium, diclofenac sodium, etodolac, felbinac, fenclofenac, fenclorac, fenclozic acid, fentiazac, furofenac, glucametacin, ibufenac, indomethacin, isofezolac, isoxepac, lonazolac, metiazinic acid, oxametacin, oxpinac, pimetacin, proglumetacin, sulindac, talmetacin, tiaramide, tiopinac, tolmetin, tolmetin sodium, zidometacin and zomepirac. Structurally related acetic 20 acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more fenamic acid derivatives, prodrug 25 esters, or pharmaceutically acceptable salts thereof. The fenamic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof comprise: enfenamic acid, etofenamate, flufenamic acid, isonixin, meclofenamic acid, meclofenamate sodium, medofenamic acid, mefenamic acid, niflumic acid, talniflumate, terofenamate, tolfenamic acid and ufenamate. Structurally related fenamic acid 30 derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In an additional specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more carboxylic acid derivatives, prodrug esters, or pharmaceutically acceptable salts thereof. The carboxylic acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof which  
5 can be used comprise: clidanac, diflunisal, flufenisal, inoridine, ketorolac and tinoridine. Structurally related carboxylic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

10 In yet another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more butyric acid derivatives, prodrug esters, or pharmaceutically acceptable salts thereof. The butyric acid derivatives, prodrug esters, and pharmaceutically acceptable salts thereof comprise:  
15 bumadizon, butibufen, fenbufen and xenbucin. Structurally related butyric acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or  
20 concurrent treatment) with any of one or more oxicams, prodrug esters, or pharmaceutically acceptable salts thereof. The oxicams, prodrug esters, and pharmaceutically acceptable salts thereof comprise: droxicam, enolicam, isoxicam, piroxicam, sudoxicam, tenoxicam and 4-hydroxyl-1,2-benzothiazine 1,1-dioxide 4-(N-phenyl)-carboxamide. Structurally related oxicams having  
25 similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In still another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more pyrazoles, prodrug esters, or  
30 pharmaceutically acceptable salts thereof. The pyrazoles, prodrug esters, and pharmaceutically acceptable salts thereof which may be used comprise:



difenamizole and eprizole. Structurally related pyrazoles having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In an additional specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment or, concurrent treatment) with any of one or more pyrazolones, prodrug esters, or pharmaceutically acceptable salts thereof. The pyrazolones, prodrug esters, and pharmaceutically acceptable salts thereof which may be used comprise: apazone, azapropazone, benzpiperylon, feprazone, mofebutazone, morazone, oxyphenbutazone, phenylbutazone, pipebuzone, propylphenazone, ramifenazone, suxibuzone and thiazolinobutazone. Structurally related pyrazolones having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more of the following: NSAIDs:  $\epsilon$ -acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, anitrazafen, antrafenine, bendazac, bendazac lysinate, benzydamine, beprozin, broperamole, bucolome, bufezolac, ciproquazone, cloximate, dazidamine, deboxamet, detomidine, difenpiramide, difenpyramide, difisalamine, ditazol, emorfazone, fanetizole mesylate, fenflumizole, floctafenine, flumizole, flunixin, fluproquazone, fopirtoline, fosfosal, guaimesal, guaiazolene, isonixirn, lefetamine HCl, leflunomide, lofemizole, lotifazole, lysin clonixinate, meseclazone, nabumetone, nictindole, nimesulide, orgotein, orpanoxin, oxaceprol, oxapadol, paranyline, perisoxal, perisoxal citrate, pifoxime, piproxen, pirazolac, pirfenidone, proquazone, proxazole, thielavin B, tiiflamizole, timegadine, tolectin, tolpadol, tryptamid and those designated by company code number such as 480156S, AA861, AD1590, AFP802, AFP860, AI77B, AP504, AU8001, BPPC, BW540C, CHINOIN 127, CN100, EB382, EL508, F1044, FK-506, GV3658, ITF182, KCNTEI6090, KME4, LA2851, MR714, MR897, MY309, ONO3144, PR823, PV102, PV108, R830, RS2131, SCR152, SH440, SIR133, SPAS510,

SQ27239, ST281, SY6001, TA60, TAI-901 (4-benzoyl-1-indancarboxylic acid), TVX2706, U60257, UR2301 and WY41770. Structurally related NSAIDs having similar analgesic and anti-inflammatory properties to the NSAIDs are also intended to be encompassed by this group.

5           In still another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment or concurrent treatment) with any of one or more corticosteroids, prodrug esters, or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation such as  
10   rheumatic diseases, graft versus host disease, and multiple sclerosis. Corticosteroids, prodrug esters, and pharmaceutically acceptable salts thereof include hydrocortisone and compounds which are derived from hydrocortisone, such as 21-acetoxypregnenolone, alclomerasone, algestone, amcinonide, beclomethasone, betamethasone, betamethasone valerate, budesonide,  
15   chloroprednisone, clobetasol, clobetasol propionate, clobetasone, clobetasone butyrate, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacon, desonide, desoximetasone, dexamethasone, diflorasone, difluocortolone, difluprednate, enoxolone, fluazacort, flucoronide, flumethasone, flumethasone pivalate, flucinolone acetonide, flunisolide, fluocinonide,  
20   fluorocinolone acetonide, fluocortin butyl, fluocortolone, fluocortolone hexanoate, difluocortolone valerate, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandenolide, formocortal, halcinonide, halometasone, halopredone acetate, hydrocortamate, hydrocortisone, hydrocortisone acetate, hydrocortisone butyrate, hydrocortisone phosphate, hydrocortisone 21-sodium  
25   succinate, hydrocortisone tebutate, mazipredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 21-diedryaminoacetate, prednisolone sodium phosphate, prednisolone sodium succinate, prednisolone sodium 21-*m*-sulfobenzoate, prednisolone sodium 21-stearoglycolate, prednisolone tebutate,  
30   prednisolone 21-trimethylacetate, prednisone, prednival, prednylidene, prednylidene 21-diethylaminoacetate, tixocortol, triamcinolone, triamcinolone

acetone, triamcinolone benetonide and triamcinolone hexacetone. Structurally related corticosteroids having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more slow-acting antirheumatic drugs (SAARDs) or disease modifying antirheumatic drugs (DMARDs), prodrug esters, or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation such as rheumatic diseases, graft versus host disease, and multiple sclerosis. SAARDs or DMARDs, prodrug esters, and pharmaceutically acceptable salts thereof comprise: allocupreide sodium, auranofin, aurothioglucose, aurothioglycanide, azathioprine, brequinar sodium, bucillamine, calcium 3-aurothio-2-propanol-1-sulfonate, chlorambucil, chloroquine, clobazart, cuproxoline, cyclophosphamide, cyclosporin, dapsone, 15-deoxyspergualin, diacerein, glucosamine, gold salts (e.g., cycloquine gold salt, gold sodium thiomalate, gold sodium thiosulfate), hydroxychloroquine, hydroxychloroquine sulfate, hydroxyurea, kebozone, levamisole, lobenzarit, melittin, 6-mercaptopurine, methotrexate, mizoribine, mycophenolate mofetil, myoral, nitrogen mustard, D-penicillamine, pyridinol imidazoles such as SKNF86002 and SB203580, rapamycin, thiols, thymopoietin and vincristine. Structurally related SAARDs or DMARDs having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

In another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more COX2 inhibitors, prodrug esters, or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation. Examples of COX2 inhibitors, prodrug esters, or pharmaceutically acceptable salts thereof include, for example, celecoxib. Structurally related COX2 inhibitors having similar analgesic and anti-inflammatory properties are also intended to be

encompassed by this group.

In still another specific embodiment, the present invention is directed to the use of an IL-1ra-R polypeptide in combination (pretreatment, post-treatment, or concurrent treatment) with any of one or more antimicrobials, prodrug esters, or pharmaceutically acceptable salts thereof for the treatment of the diseases and disorders recited herein, including acute and chronic inflammation. Antimicrobials include, for example, the broad classes of penicillins, cephalosporins and other beta-lactams, aminoglycosides, azoles, quinolones, macrolides, rifamycins, tetracyclines, sulfonamides, lincosamides and polymyxins. The penicillins include, but are not limited to, penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, floxacillin, ampicillin, ampicillin/sulbactam, amoxicillin, amoxicillin/clavulanate, hetacillin, cyclacillin, bacampicillin, carbenicillin, carbenicillin indanyl, ticarcillin, ticarcillin/clavulanate, azlocillin, mezlocillin, peperacillin, and mecillinam. The cephalosporins and other beta-lactams include, but are not limited to, cephalothin, cephapirin, cephalexin, cephadrine, cefazolin, cefadroxil, cefaclor, cefamandole, cefotetan, cefoxitin, ceruroxime, cefonicid, ceforadine, cefixime, cefotaxime, moxalactam, ceftizoxime, ceftriaxone, cephaloperazone, ceftazidime, imipenem and aztreonam. The aminoglycosides include, but are not limited to, streptomycin, gentamicin, tobramycin, amikacin, netilmicin, kanamycin and neomycin. The azoles include, but are not limited to, fluconazole. The quinolones include, but are not limited to, nalidixic acid, norfloxacin, enoxacin, ciprofloxacin, ofloxacin, sparfloxacin and temafloxacin. The macrolides include, but are not limited to, erythromycin, spiramycin and azithromycin. The rifamycins include, but are not limited to, rifampin. The tetracyclines include, but are not limited to, spicycline, chlortetracycline, clomocycline, demeclocycline, deoxycycline, guamecycline, lymecycline, meclocycline, methacycline, minocycline, oxytetracycline, penimepicycline, pipacycline, rolitetracycline, sancycline, senociclin and tetracycline. The sulfonamides include, but are not limited to, sulfanilamide, sulfamethoxazole, sulfacetamide, sulfadiazine, sulfisoxazole and co-trimoxazole (trimethoprim/sulfamethoxazole). The lincosamides include, but are not limited

to, clindamycin and lincomycin. The polymyxins (polypeptides) include, but are not limited to, polymyxin B and colistin.

Agonists or antagonists of IL-1RA-R polypeptide function may be used (simultaneously or sequentially) in combination with one or more cytokines, growth factors, antibiotics, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the condition being treated.

Other diseases caused by or mediated by undesirable levels of one or more of IL-1, IL-1ra, or IL-1ra-R polypeptide are encompassed within the scope of the invention. Undesirable levels include excessive levels of IL-1, IL-1ra, or IL-1ra-R polypeptide and sub-normal levels of IL-1, IL-1ra, or IL-1ra-R polypeptide.

#### Uses of IL-1ra-R Nucleic Acids and Polypeptides

Nucleic acid molecules of the invention (including those that do not themselves encode biologically active polypeptides) may be used to map the locations of the IL-1ra-R gene and related genes on chromosomes. Mapping may be done by techniques known in the art, such as PCR amplification and *in situ* hybridization.

IL-1ra-R nucleic acid molecules (including those that do not themselves encode biologically active polypeptides), may be useful as hybridization probes in diagnostic assays to test, either qualitatively or quantitatively, for the presence of an IL-1ra-R nucleic acid molecule in mammalian tissue or bodily fluid samples.

Other methods may also be employed where it is desirable to inhibit the activity of one or more IL-1ra-R polypeptides. Such inhibition may be effected by nucleic acid molecules that are complementary to and hybridize to expression control sequences (triple helix formation) or to IL-1ra-R mRNA. For example, antisense DNA or RNA molecules, which have a sequence that is complementary to at least a portion of an IL-1ra-R gene can be introduced into the cell. Antisense probes may be designed by available techniques using the sequence of the IL-1ra-R gene disclosed herein. Typically, each such antisense molecule will be complementary to the start site (5' end) of each selected IL-1ra-R gene. When the antisense molecule then hybridizes to the corresponding IL-1ra-R mRNA,

translation of this mRNA is prevented or reduced. Anti-sense inhibitors provide information relating to the decrease or absence of an IL-1ra-R polypeptide in a cell or organism.

Alternatively, gene therapy may be employed to create a dominant-negative inhibitor of one or more IL-1ra-R polypeptides. In this situation, the DNA encoding a mutant polypeptide of each selected IL-1ra-R polypeptide can be prepared and introduced into the cells of a patient using either viral or non-viral methods as described herein. Each such mutant is typically designed to compete with endogenous polypeptide in its biological role.

In addition, an IL-1ra-R polypeptide, whether biologically active or not, may be used as an immunogen, that is, the polypeptide contains at least one epitope to which antibodies may be raised. Selective binding agents that bind to an IL-1ra-R polypeptide (as described herein) may be used for *in vivo* and *in vitro* diagnostic purposes, including, but not limited to, use in labeled form to detect the presence of IL-1ra-R polypeptide in a body fluid or cell sample. The antibodies may also be used to prevent, treat, or diagnose a number of diseases and disorders, including those recited herein. The antibodies may bind to an IL-1ra-R polypeptide so as to diminish or block at least one activity characteristic of an IL-1ra-R polypeptide, or may bind to a polypeptide to increase at least one activity characteristic of an IL-1ra-R polypeptide (including by increasing the pharmacokinetics of the IL-1ra-R polypeptide).

The IL-1ra-R polypeptides of the present invention can be used to clone IL-1ra-R polypeptide receptors, using an expression cloning strategy. Radiolabeled (<sup>125</sup>Iodine) IL-1ra-R polypeptide or affinity/activity-tagged IL-1ra-R polypeptide (such as an Fc fusion or an alkaline phosphatase fusion) can be used in binding assays to identify a cell type or cell line or tissue that expresses IL-1ra-R polypeptide receptors. RNA isolated from such cells or tissues can be converted to cDNA, cloned into a mammalian expression vector, and transfected into mammalian cells (such as COS or 293 cells) to create an expression library. A radiolabeled or tagged IL-1ra-R polypeptide can then be used as an affinity ligand to identify and isolate from this library the subset of cells that express the

IL-1ra-R polypeptide receptors on their surface. DNA can then be isolated from these cells and transfected into mammalian cells to create a secondary expression library in which the fraction of cells expressing IL-1ra-R polypeptide receptors is many-fold higher than in the original library. This enrichment process can be repeated iteratively until a single recombinant clone containing an IL-1ra-R polypeptide receptor is isolated. Isolation of the IL-1ra-R polypeptide receptors is useful for identifying or developing novel agonists and antagonists of the IL-1ra-R polypeptide signaling pathway. Such agonists and antagonists include soluble IL-1ra-R polypeptide receptors, anti-IL-1ra-R polypeptide receptor antibodies, small molecules, or antisense oligonucleotides, and they may be used for treating, preventing, or diagnosing one or more of the diseases or disorders described herein.

A deposit of cDNA encoding human IL-1ra-R polypeptide, having Accession No. PTA-1423, was made with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209 on February 29, 2000.

The following examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

#### Example 1: Cloning of the Human IL-1ra-R Polypeptide Gene

Generally, materials and methods as described in Sambrook *et al. supra* were used to clone and analyze the gene encoding human IL-1ra-R polypeptide.

To isolate cDNA sequences encoding human IL-1ra-R polypeptide, a search of a proprietary database (Amgen, Thousand Oaks, CA) was performed using clone shos1-00003-d1 as a query sequence. A 423 bp sequence identified in this manner was found to share 41% identity to the query sequence at the amino acid level. The region of highest homology within this sequence was used to design gene specific oligonucleotides for the identification of cDNA sources and the generation of cDNA clones, using various PCR strategies.

A number of cDNA libraries were analyzed in amplification reactions containing 10ng of cDNA library template DNA, 10 pmol each of the amplimers

2349-98 (5'-C-A-C-A-C-G-C-T-T-C-A-C-C-T-T-C-T-T-T-C-C-A-G-3'; SEQ ID NO: 20) and 2349-99 (5'-T-A-A-A-A-C-T-T-G-G-T-A-C-G-G-G-C-T-G-A-G-G-G-3'; SEQ ID NO: 21), and Ready-To-Go PCR beads (Amersham-Pharmacia, Piscataway, NJ) (Pharmacia, Piscataway, NJ), in a total reaction volume of 25  $\mu$ l.

- 5 Reactions were performed at 95°C for 5 minutes for one cycle; 95°C for 15 seconds, 63°C for 15 seconds, and 72°C for 1 minute for 30 cycles; and 72°C for 10 minutes for one cycle. A PCR product of the expected size (153 bp) was identified in a number of cDNA libraries, including human fetal scalp (oligo-dT primed) and human placenta (oligo-dT primed and random primed).

- 10 The fetal scalp and placenta cDNA libraries were prepared as follows. Total RNA was extracted from human fetal scalp and from human placenta using standard RNA extraction procedures and poly-A<sup>+</sup> RNA was selected from this total RNA using standard procedures. Random primed or oligo-dT primed cDNA was synthesized from this poly-A<sup>+</sup> RNA using the Superscript Plasmid System for  
15 cDNA Synthesis and Plasmid Cloning kit (Gibco-BRL), according to the manufacturer's suggested protocols, or other suitable procedure. The resulting cDNA was digested with appropriate restriction enzymes and was then ligated into pSPORT-1, or other suitable cloning vector. Ligation products were transformed into *E. coli* using standard techniques, and bacterial transformants  
20 were selected on culture plates containing either ampicillin, tetracycline, kanamycin, or chloramphenicol. The cDNA library consisted of all, or a subset, of these transformants.

- Both 5'RACE and 3'RACE reactions were performed in order to generate the full-length cDNA sequence for IL-1ra-R polypeptide. To isolate cDNA  
25 sequences corresponding to the 5' end of the cDNA sequence for IL-1ra-R polypeptide, 5'RACE was performed using 10 ng of an oligo-dT-primed human fetal scalp cDNA library in pSPORT1 and the primers 1572-36 (5'-G-T-G-T-G-G-A-A-T-T-G-T-G-A-G-C-G-G-A-T-A-A-C-3'; SEQ ID NO: 22) and 2349-99. Reactions were performed at 94°C for 1 minute for one cycle; 94°C for 5 seconds  
30 and 72°C for 5 minutes for 5 cycles; 94°C for 5 seconds, 70°C for 10 seconds, and 72°C for 3 minutes for 5 cycles; 94°C for 5 seconds, 68°C for 10 seconds, and



72°C for 3 minutes for 25 cycles; and 72°C for 7 minutes for one cycle. Nested PCR was performed using a portion of the 5'RACE amplification product and the primers 2328-91 (5'-C-T-A-T-G-A-C-C-A-T-G-A-T-T-A-C-G-C-C-A-A-G-C-3'; SEQ ID NO: 23) and 2351-47 (5'-G-C-T-G-T-A-C-T-G-G-C-T-G-C-T-G-G-G-G-C-3'; SEQ ID NO: 24). Nested PCR was performed at 94°C for 1 minute for one cycle; 94°C for 5 seconds and 72°C for 5 minutes for 5 cycles; 94°C for 5 seconds, 70°C for 10 seconds, and 72°C for 3 minutes for 5 cycles; 94°C for 5 seconds, 68°C for 10 seconds, and 72°C for 3 minutes for 25 cycles; and 72°C for 7 minutes for one cycle.

To isolate cDNA sequences corresponding to the 3' end of the cDNA sequence for IL-1ra-R polypeptide, 3'RACE was performed using 10 ng of an oligo-dT-primed human fetal scalp cDNA library in pSPORT1 and the primers 2351-48 (5'-C-C-T-T-C-A-G-G-C-T-T-G-A-G-G-C-T-G-C-T-G-3'; SEQ ID NO: 25) and 2329-93 (5'-C-G-G-G-C-C-T-C-T-T-C-G-C-T-A-T-T-A-C-G-C-3'; SEQ ID NO: 26). Reactions were performed at 94°C for 1 minute for one cycle; 94°C for 5 seconds and 72°C for 5 minutes for 5 cycles; 94°C for 5 seconds, 70°C for 10 seconds, and 72°C for 3 minutes for 5 cycles; 94°C for 5 seconds, 68°C for 10 seconds, and 72°C for 3 minutes for 25 cycles; and 72°C for 7 minutes for one cycle. Nested PCR was performed using a portion of the 3'RACE amplification product and the primers 2363-04 (5'-C-C-T-G-G-C-T-G-G-T-T-C-C-T-G-T-G-T-G-G-C-3'; SEQ ID NO: 27) and 2329-94 (5'-T-G-G-C-G-A-A-A-G-G-G-G-A-T-G-T-G-C-T-G-3'; SEQ ID NO: 28). Nested PCR was performed at 95°C for 1 minute for one cycle; 95°C for 30 seconds and 68°C for 1 minute for 30 cycles; and 68°C for 7 minutes for one cycle. The full-length cDNA sequence for IL-1ra-R polypeptide was assembled from the resulting collection of 5'RACE and 3'RACE clones.

The 5' portion of the cDNA sequence for IL-1ra-R polypeptide was confirmed by independently isolating cDNA sequences from a human placenta Marathon™ cDNA library (Clontech). To isolate cDNA sequences corresponding to the 5' end of the cDNA sequence for IL-1ra-R polypeptide, 5'RACE was performed using 1 ng of human placenta cDNA and the primers AP-1 (5'-C-C-A-

T-C-C-T-A-A-T-A-C-G-A-C-T-C-A-C-T-A-T-A-G-G-G-C-3'; SEQ ID NO: 29; Clontech) and 2353-87 (5'-C-C-T-T-G-G-T-G-A-G-C-T-G-T-A-C-T-G-G-C-T-G-3'; SEQ ID NO: 30). Reactions were performed at 94°C for 2 minutes for one cycle; 94°C for 5 seconds and 72°C for 1.5 minutes for 5 cycles; 94°C for 5 seconds and 70°C for 1.5 minutes for 5 cycles; 94°C for 5 seconds and 68°C for 1.5 minutes for 25 cycles; and 86°C for 7 minutes for one cycle. Nested PCR was performed using a portion of the 5'RACE amplification product and the primers AP-1 and 2349-52 (5'-C-C-G-G-G-C-C-A-C-A-C-A-G-G-A-A-C-C-A-3'; SEQ ID NO: 31). Nested PCR was performed at 94°C for 2 minutes for one cycle; 94°C for 10 seconds and 68°C for 1.5 minutes for 30 cycles; and 68°C for 7 minutes for one cycle.

Sequence analysis of the predicted cDNA sequence for human IL-1ra-R polypeptide indicated that the gene comprises a 456 bp open reading frame encoding a protein of 152 amino acids (Figures 1A-1B). A sequence variant of the human IL-1ra-R polypeptide was also identified. Sequence analysis of this variant indicated that the gene for this variant also comprises a 456 bp open reading frame encoding a protein of 152 amino acids (Figures 2A-2B). The nucleotide sequence of this variant differs from that of the human IL-1ra-R gene at two nucleotide positions.

Figures 4A-4B illustrate the amino acid sequence alignment of human IL-1 $\alpha$  (IL-1\_alpha; SEQ ID NO: 7), human IL-1 $\beta$  (IL-1\_beta; SEQ ID NO: 8), human IL-1 receptor antagonist (IL-1RA; SEQ ID NO: 9), human IL-1 $\delta$  (IL-1\_delta; SEQ ID NO: 10), human IL-1ra-R polypeptide (IL-1ra-R; SEQ ID NO: 2), human Tango-77 (Tango-77; SEQ ID NO: 11), human Zilla4 (Zilla4; SEQ ID NO: 12), human IL-1 $\zeta$  (IL-1\_zeta; SEQ ID NO: 13), human IL-1 receptor antagonist  $\beta$  (IL-1RA\_beta; SEQ ID NO: 14), human SPOIL II (Spoil\_II; SEQ ID NO: 15), human IL-1 $\epsilon$  (IL-1\_epsilon; SEQ ID NO: 16), and human IL-1 $\eta$  (IL-1\_eta; SEQ ID NO: 17). Figure 5 schematically illustrates the phylogenetic relationship of the IL-1ra gene family.

#### Example 2: Cloning of the Human IL-1ra-R Polypeptide Gene Splice Variant

Generally, materials and methods as described in Sambrook *et al. supra* were used to clone and analyze the gene encoding a human IL-1ra-R polypeptide splice variant.

Full-length cDNA sequences encoding a splice variant of human IL-1ra-R polypeptide, were isolated from a human placenta cDNA library, which was prepared as follows. Total RNA was extracted from human placenta using standard RNA extraction procedures and poly-A<sup>+</sup> RNA was selected from this total RNA using standard procedures. Oligo-dT primed cDNA was synthesized from this poly-A<sup>+</sup> RNA using the Superscript Plasmid System for cDNA Synthesis and Plasmid Cloning kit (Gibco-BRL), according to the manufacturer's suggested protocols. The resulting cDNA was digested with Not I and then fractionated by electrophoresis on a 0.8% agarose gel. Fragments of 0.8 to 1.6 kb were isolated, purified, and then ligated into pSPORT-1. Ligation products were transformed into *E. coli* using standard techniques, and bacterial transformants were selected on culture plates containing ampicillin. The resulting transformants were pooled to generate the cDNA library and plasmid DNA was prepared from 12 pools (each containing approximately 80,000 colonies) of this cDNA library.

Each of the cDNA library pools was analyzed in amplification reactions containing 10ng of template DNA, the amplimers 2349-98 and 2349-99 at a concentration of 0.4  $\mu$ M each, and Ready-To-Go PCR beads, in a total reaction volume of 25  $\mu$ l. Reactions were performed at 94°C for 5 minutes for one cycle; 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 1 minute for 30 cycles; and 72°C for 10 minutes for one cycle. The cDNA library pools were also analyzed in amplification reactions containing 10ng of template DNA, the amplimers 2349-51 (5'-A-A-G-A-G-G-C-C-A-C-A-C-G-C-T-T-C-A-C-C-T-T-C-T-3'; SEQ ID NO: 32) and 2349-52 at a concentration of 0.4  $\mu$ M each, and Ready-To-Go PCR beads, in a total reaction volume of 25  $\mu$ l. Reactions were performed at 94°C for 5 minutes for one cycle; 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 1 minute for 30 cycles; and 72°C for 10 minutes for one cycle. The PCR products obtained in these reactions were then analyzed on agarose gels.

Plasmid DNA from three pools yielded PCR products having the expected

size, and  $3 \times 10^5$  clones from each of these positive pools were screened by PCR using the amplimers 2349-98 and 2349-99. One of the PCR products (zhvt-000329) generated by PCR was labeled with  $^{32}\text{P}$ -dCTP and used as a probe to re-screen the positive cDNA library pools. Bacteria colonies were plated (at  
5 approximately 50,000 per 150 mm plate) and then lifted onto nitrocellulose filters. Filters were prehybridized in ExpressHyb hybridization solution (Clontech) for 30 minutes at  $68^\circ\text{C}$ , and then hybridized in the same solution with the addition of labeled probe at  $68^\circ\text{C}$  overnight. Following hybridization, the filters were washed twice for 10 minutes at room temperature in 2X SSC and 0.05% SDS, and then  
10 twice for 30 minutes at  $68^\circ\text{C}$  in 0.1X SSC and 0.1% SDS. Filters were then exposed to autoradiography with intensifying screens at  $-80^\circ\text{C}$  for 2 hours. A positive clone (RDS#199918503) containing an insert of approximately 1.2 kb was identified from one of the cDNA library pools. A second positive clone (RDS#199918501) containing insert size about 0.8 kb was identified from a  
15 separate cDNA library pool. Plasmid DNA was prepared from each of these clones and the sequence was analyzed.

Sequence analysis of the predicted cDNA sequence for one of these clones indicated that the cDNA encoded a variant of human IL-1ra-R polypeptide (see Example 1). The gene encoding this variant comprises a 513 bp open reading  
20 frame encoding a protein of 171 amino acids (Figure 3).

Sequence analysis of the human genomic sequence containing the IL-1ra-R gene (Genbank Accession No. AC016724, contig. fragment 76649-96342) indicated that the first exon of the IL-1ra-R gene (see Example 1) lies 4.1 kb downstream of the last exon of IL-1 Omega (Genbank Accession No. Z300050).  
25 The close proximity of these two genes in the genome suggests that the present polypeptide might arise from splicing (or a fusion) of the first two exons of IL-1 Omega onto the second exon of the IL-1ra-R gene, or a variant thereof (see Example 1). This variant of the IL-1ra-R polypeptide may therefore be an IL-1ra-R splice variant, fusion protein, or the like. Juxtaposition of the second exon in  
30 IL-1 Omega and the second exon of the IL-1ra-R gene (or a variant thereof) results in a sequence that encodes a protein with an N-terminus that appears to

function as a signal peptide. Figure 6 schematically illustrates the relationship between human IL-1ra-R polypeptide (Mature CS329), the sequence variant of human IL-1ra-R polypeptide (Mature CS329 Variant protein), and the splice variant of human IL-1ra-R polypeptide (Omega 329 protein).

5

### Example 3: Cloning of the Murine IL-1ra-R Polypeptide Gene

Generally, materials and methods as described in Sambrook *et al. supra* were used to clone and analyze the gene encoding murine IL-1ra-R polypeptide.

To isolate cDNA sequences encoding murine IL-1ra-R polypeptide, PCR  
10 was performed using a day 7 mouse embryo cDNA library template and the amplimers 2557-95 (5'-A-A-G-C-C-T-T-T-T-T-C-T-T-C-T-T-T-G-C-C-T-C-A-G-T-G-3'; SEQ ID NO: 33) and 2557-96 (5'-T-G-C-C-A-T-T-T-A-A-T-G-T-A-A-C-A-C-G-G-T-C-A-C-A-G-3'; SEQ ID NO: 34) and standard techniques.

Sequence analysis of the predicted cDNA sequence for murine IL-1ra-R  
15 polypeptide indicated that the gene comprises a 456 bp open reading frame encoding a protein of 152 amino acids (Figure 7). Figure 8 illustrates the amino acid sequence alignment of human IL-1ra-R polypeptide (huIL-1ra-R; SEQ ID NO: 2) and murine IL-1ra-R polypeptide (muIL-1ra-R; SEQ ID NO: 36). Figures 9A-9I illustrate the genomic nucleotide sequence for the murine IL-1ra-R gene  
20 (SEQ ID NO: 37). The locations of the coding portions of exons 1-4 are indicated (underline).

### Example 4: IL-1ra-R mRNA Expression

Expression of IL-1ra-R mRNA was examined by PCR in amplification  
25 reactions containing 10ng of cDNA library template DNA, 10 pmol of the amplimers 2349-98 and 2349-99, and Ready-To-Go PCR beads, in a total reaction volume of 25 µl. Reactions were performed at 95°C for 5 minutes for one cycle; 95°C for 15 seconds, 63°C for 15 seconds, and 72°C for 1 minute for 30 cycles; and 72°C for 10 minutes for one cycle. A PCR product of the expected size (153  
30 bp) was identified in a number of cDNA libraries, including human fetal scalp, human fetal eye, human gall bladder, and human placenta. IL-1ra-R mRNA

expression was also detected in fetal eye and fetal spleen by RT-PCR using the amplimers 2349-51 and 2349-52 and the Titan system (Boehringer). These reactions were performed at 55°C for 30 minutes for one cycle; 94°C for 15 seconds, 64°C for 15 seconds, and 68°C for 50 seconds (with an increase of 2  
5 seconds per cycle) for 30 cycles; and 68°C for 7 minutes for one cycle.

Expression of IL-1ra-R mRNA was examined by Northern blot analysis using human and mouse RNA blots (Clontech). Blots were first prehybridized for 30 minutes at 65°C in Pre-Hyb Solution (Amersham) and then probed overnight at  
10 65°C in the same solution containing 25 ng of a <sup>32</sup>P-labeled probe corresponding to nucleotides 1-474 of the cDNA sequence encoding IL-1ra-R polypeptide. The probe was labeled using a Redi Prime II kit (Pharmacia). Following hybridization, blots were washed twice for 1 hour in 6X SSC and 0.1% SDS, twice for 1 hour in 2X SSC and 0.1% SDS, twice for 30 minutes in 0.2X SSC and  
15 0.1% SDS, and then were rinsed in 2X SSC. Following overnight exposure of the Northern blots in a phosphoimager, a transcript of approximately 1.6 kb was detected in mouse skeletal muscle (Figure 10A) and a transcript of approximately 2.9 kb was detected in human pancreas and peripheral blood leukocytes (Figure 10B and 10C). In an analysis of dot blots (Clontech) using the same probe, IL-  
20 1ra-R mRNA was detected in murine skeletal muscle, submaxillar gland, and epididymis.

The expression of IL-1ra-R mRNA is localized by *in situ* hybridization. A panel of normal embryonic and adult mouse tissues is fixed in 4%  
25 paraformaldehyde, embedded in paraffin, and sectioned at 5 µm. Sectioned tissues are permeabilized in 0.2 M HCl, digested with Proteinase K, and acetylated with triethanolamine and acetic anhydride. Sections are prehybridized for 1 hour at 60°C in hybridization solution (300 mM NaCl, 20 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1X Denhardt's solution, 0.2% SDS, 10 mM DTT, 0.25 mg/ml  
30 tRNA, 25 µg/ml polyA, 25 µg/ml polyC and 50% formamide) and then hybridized overnight at 60°C in the same solution containing 10% dextran and 2 x 10<sup>4</sup> cpm/µl

of a  $^{33}\text{P}$ -labeled antisense riboprobe complementary to the human IL-1ra-R gene. The riboprobe is obtained by *in vitro* transcription of a clone containing human IL-1ra-R cDNA sequences using standard techniques.

Following hybridization, sections are rinsed in hybridization solution, treated with RNaseA to digest unhybridized probe, and then washed in 0.1X SSC at 55°C for 30 minutes. Sections are then immersed in NTB-2 emulsion (Kodak, Rochester, NY), exposed for 3 weeks at 4°C, developed, and counterstained with hematoxylin and eosin. Tissue morphology and hybridization signal are simultaneously analyzed by darkfield and standard illumination for brain (one sagittal and two coronal sections), gastrointestinal tract (esophagus, stomach, duodenum, jejunum, ileum, proximal colon, and distal colon), pituitary, liver, lung, heart, spleen, thymus, lymph nodes, kidney, adrenal, bladder, pancreas, salivary gland, male and female reproductive organs (ovary, oviduct, and uterus in the female; and testis, epididymus, prostate, seminal vesicle, and vas deferens in the male), BAT and WAT (subcutaneous, peri-renal), bone (femur), skin, breast, and skeletal muscle.

#### Example 5: Production of IL-1ra-R Polypeptides

##### A. Expression of IL-1ra-R Polypeptides in Bacteria

PCR is used to amplify template DNA sequences encoding an IL-1ra-R polypeptide using primers corresponding to the 5' and 3' ends of the sequence. The amplified DNA products may be modified to contain restriction enzyme sites to allow for insertion into expression vectors. PCR products are gel purified and inserted into expression vectors using standard recombinant DNA methodology. An exemplary vector, such as pAMG21 (ATCC no. 98113) containing the lux promoter and a gene encoding kanamycin resistance is digested with Bam HI and Nde I for directional cloning of inserted DNA. The ligated mixture is transformed into an *E. coli* host strain by electroporation and transformants are selected for kanamycin resistance. Plasmid DNA from selected colonies is isolated and subjected to DNA sequencing to confirm the presence of the insert.

Transformed host cells are incubated in 2xYT medium containing 30

µg/mL kanamycin at 30°C prior to induction. Gene expression is induced by the addition of N-(3-oxohexanoyl)-dl-homoserine lactone to a final concentration of 30 ng/mL followed by incubation at either 30°C or 37°C for six hours. The expression of IL-1ra-R polypeptide is evaluated by centrifugation of the culture, resuspension and lysis of the bacterial pellets, and analysis of host cell proteins by SDS-polyacrylamide gel electrophoresis.

Inclusion bodies containing IL-1ra-R polypeptide are purified as follows. Bacterial cells are pelleted by centrifugation and resuspended in water. The cell suspension is lysed by sonication and pelleted by centrifugation at 195,000 xg for 5 to 10 minutes. The supernatant is discarded, and the pellet is washed and transferred to a homogenizer. The pellet is homogenized in 5 mL of a Percoll solution (75% liquid Percoll and 0.15 M NaCl) until uniformly suspended and then diluted and centrifuged at 21,600 xg for 30 minutes. Gradient fractions containing the inclusion bodies are recovered and pooled. The isolated inclusion bodies are analyzed by SDS-PAGE.

A single band on an SDS polyacrylamide gel corresponding to *E. coli*-produced IL-1ra-R polypeptide is excised from the gel, and the N-terminal amino acid sequence is determined essentially as described by Matsudaira *et al.*, 1987, *J. Biol. Chem.* 262:10-35.

#### B. Expression of IL-1ra-R Polypeptide in Mammalian Cells

PCR is used to amplify template DNA sequences encoding an IL-1ra-R polypeptide using primers corresponding to the 5' and 3' ends of the sequence. The amplified DNA products may be modified to contain restriction enzyme sites to allow for insertion into expression vectors. PCR products are gel purified and inserted into expression vectors using standard recombinant DNA methodology. An exemplary expression vector, pCEP4 (Invitrogen, Carlsbad, CA), that contains an Epstein-Barr virus origin of replication, may be used for the expression of IL-1ra-R polypeptides in 293-EBNA-1 cells. Amplified and gel purified PCR products are ligated into pCEP4 vector and introduced into 293-EBNA cells by lipofection. The transfected cells are selected in 100 µg/mL hygromycin and the



resulting drug-resistant cultures are grown to confluence. The cells are then cultured in serum-free media for 72 hours. The conditioned media is removed and IL-1ra-R polypeptide expression is analyzed by SDS-PAGE.

- IL-1ra-R polypeptide expression may be detected by silver staining.
- 5 Alternatively, IL-1ra-R polypeptide is produced as a fusion protein with an epitope tag, such as an IgG constant domain or a FLAG epitope, which may be detected by Western blot analysis using antibodies to the peptide tag.

- IL-1ra-R polypeptides may be excised from an SDS-polyacrylamide gel, or IL-1ra-R fusion proteins are purified by affinity chromatography to the epitope tag, and subjected to N-terminal amino acid sequence analysis as described herein.
- 10

#### C. Expression and Purification of IL-1ra-R Polypeptide in Mammalian Cells

IL-1ra-R polypeptide expression constructs are introduced into 293 EBNA or CHO cells using either a lipofection or calcium phosphate protocol.

- 15 To conduct functional studies on the IL-1ra-R polypeptides that are produced, large quantities of conditioned media are generated from a pool of hygromycin selected 293 EBNA clones. The cells are cultured in 500 cm Nunc Triple Flasks to 80% confluence before switching to serum free media a week prior to harvesting the media. Conditioned media is harvested and frozen at
- 20 -20°C until purification.

- Conditioned media is purified by affinity chromatography as described below. The media is thawed and then passed through a 0.2  $\mu$ m filter. A Protein G column is equilibrated with PBS at pH 7.0, and then loaded with the filtered media. The column is washed with PBS until the absorbance at  $A_{280}$  reaches a
- 25 baseline. IL-1ra-R polypeptide is eluted from the column with 0.1 M Glycine-HCl at pH 2.7 and immediately neutralized with 1 M Tris-HCl at pH 8.5. Fractions containing IL-1ra-R polypeptide are pooled, dialyzed in PBS, and stored at -70°C.

- For Factor Xa cleavage of the human IL-1ra-R polypeptide-Fc fusion
- 30 polypeptide, affinity chromatography-purified protein is dialyzed in 50 mM Tris-HCl, 100 mM NaCl, 2 mM  $\text{CaCl}_2$  at pH 8.0. The restriction protease Factor Xa is

added to the dialyzed protein at 1/100 (w/w) and the sample digested overnight at room temperature.

Example 6: Production of Anti-IL-1ra-R Polypeptide Antibodies

5           Antibodies to IL-1ra-R polypeptides may be obtained by immunization with purified protein or with IL-1ra-R peptides produced by biological or chemical synthesis. Suitable procedures for generating antibodies include those described in Hudson and Bay, *Practical Immunology* (2nd ed., Blackwell Scientific Publications).

10           In one procedure for the production of antibodies, animals (typically mice or rabbits) are injected with an IL-1ra-R antigen (such as an IL-1ra-R polypeptide), and those with sufficient serum titer levels as determined by ELISA are selected for hybridoma production. Spleens of immunized animals are collected and prepared as single cell suspensions from which splenocytes are  
15           recovered. The splenocytes are fused to mouse myeloma cells (such as Sp2/0-Ag14 cells), are first incubated in DMEM with 200 U/mL penicillin, 200 µg/mL streptomycin sulfate, and 4 mM glutamine, and are then incubated in HAT selection medium (hypoxanthine, aminopterin, and thymidine). After selection, the tissue culture supernatants are taken from each fusion well and tested for anti-  
20           IL-1ra-R antibody production by ELISA.

          Alternative procedures for obtaining anti-IL-1ra-R antibodies may also be employed, such as the immunization of transgenic mice harboring human Ig loci for production of human antibodies, and the screening of synthetic antibody libraries, such as those generated by mutagenesis of an antibody variable domain.

25

          Antibodies to IL-1ra-R polypeptides were obtained by immunizing New Zealand White rabbits with full-length IL-1ra-R polypeptide produced in and isolated from *E. coli*. Crude polyclonal immune serum was collected and used in immunoprecipitation analysis using recombinant IL-1ra-R polypeptide (Figures  
30           11A-11B, lanes 1, 4, and 7), recombinant IL-1ra-R polypeptide variant (Figures 11A-11B, lanes 2, 5, and 8), and recombinant IL-1ra (Figures 11A-11B; lanes 3,

6, and 9). Gels were prepared by loading either 10 ng (Figure 11A, lanes 4-6) or 0.6  $\mu$ g (Figure 11B, lanes 7-9) of recombinant polypeptide directly onto an 18% Tris-glycine gel or by immunoprecipitating 1  $\mu$ g of recombinant polypeptide with 0.2  $\mu$ l of crude antiserum (Figure 11A, lanes 1-3) prior to loading the sample on the gel. Following SDS-PAGE separation, gels were blotted onto PVDF membranes and developed using a 1:1000 dilution of crude anti-IL-1ra-R serum followed by HRP-conjugated Protein A and ECL detection. The gel shown in Figure 11B was stained with Gelcode Blue (Pierce) following blotting. Antibodies to IL-1ra-R polypeptide were shown to detect both membrane-immobilized IL-1ra-R polypeptide and IL-1ra-R polypeptide variant, but not IL-1ra. In solution, IL-1ra-R polypeptide was more efficiently recognized than the IL-1ra-R polypeptide variant.

#### Example 7: Expression of IL-1ra-R Polypeptide in Transgenic Mice

To assess the biological activity of IL-1ra-R polypeptide, a construct encoding an IL-1ra-R polypeptide/Fc fusion protein under the control of a liver specific ApoE promoter is prepared. The delivery of this construct is expected to cause pathological changes that are informative as to the function of IL-1ra-R polypeptide. Similarly, a construct containing the full-length IL-1ra-R polypeptide under the control of the beta actin promoter is prepared. The delivery of this construct is expected to result in ubiquitous expression.

To generate these constructs, PCR is used to amplify template DNA sequences encoding an IL-1ra-R polypeptide using primers that correspond to the 5' and 3' ends of the desired sequence and which incorporate restriction enzyme sites to permit insertion of the amplified product into an expression vector. Following amplification, PCR products are gel purified, digested with the appropriate restriction enzymes, and ligated into an expression vector using standard recombinant DNA techniques. For example, amplified IL-1ra-R polypeptide sequences can be cloned into an expression vector under the control of the human  $\beta$ -actin promoter as described by Graham *et al.*, 1997, *Nature Genetics*, 17:272-74 and Ray *et al.*, 1991, *Genes Dev.* 5:2265-73.

Following ligation, reaction mixtures are used to transform an *E. coli* host strain by electroporation and transformants are selected for drug resistance. Plasmid DNA from selected colonies is isolated and subjected to DNA sequencing to confirm the presence of an appropriate insert and absence of mutation. The IL-1ra-R polypeptide expression vector is purified through two rounds of CsCl density gradient centrifugation, cleaved with a suitable restriction enzyme, and the linearized fragment containing the IL-1ra-R polypeptide transgene is purified by gel electrophoresis. The purified fragment is resuspended in 5 mM Tris, pH 7.4, and 0.2 mM EDTA at a concentration of 2 mg/mL.

Single-cell embryos from BDF1 x BDF1 bred mice are injected as described (PCT Pub. No. WO 97/23614). Embryos are cultured overnight in a CO<sub>2</sub> incubator and 15-20 two-cell embryos are transferred to the oviducts of a pseudopregnant CD1 female mice. Offspring obtained from the implantation of microinjected embryos are screened by PCR amplification of the integrated transgene in genomic DNA samples as follows. Ear pieces are digested in 20 mL ear buffer (20 mM Tris, pH 8.0, 10 mM EDTA, 0.5% SDS, and 500 mg/mL proteinase K) at 55°C overnight. The sample is then diluted with 200 mL of TE, and 2 mL of the ear sample is used in a PCR reaction using appropriate primers.

At 8 weeks of age, transgenic founder animals and control animals are sacrificed for necropsy and pathological analysis. Portions of spleen are removed and total cellular RNA isolated from the spleens using the Total RNA Extraction Kit (Qiagen) and transgene expression determined by RT-PCR. RNA recovered from spleens is converted to cDNA using the SuperScript™ Preamplification System (Gibco-BRL) as follows. A suitable primer, located in the expression vector sequence and 3' to the IL-1ra-R polypeptide transgene, is used to prime cDNA synthesis from the transgene transcripts. Ten mg of total spleen RNA from transgenic founders and controls is incubated with 1 mM of primer for 10 minutes at 70°C and placed on ice. The reaction is then supplemented with 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 10 mM of each dNTP, 0.1 mM DTT, and 200 U of SuperScript II reverse transcriptase. Following incubation for 50 minutes at 42°C, the reaction is stopped by heating for 15 minutes at 72°C and

digested with 2U of RNase H for 20 minutes at 37°C. Samples are then amplified by PCR using primers specific for IL-1ra-R polypeptide.

Example 8: Biological Activity of IL-1ra-R Polypeptide in Transgenic Mice

5           Prior to euthanasia, transgenic animals are weighed, anesthetized by isofluorane and blood drawn by cardiac puncture. The samples are subjected to hematology and serum chemistry analysis. Radiography is performed after terminal exsanguination. Upon gross dissection, major visceral organs are subject to weight analysis.

10           Following gross dissection, tissues (*i.e.*, liver, spleen, pancreas, stomach, the entire gastrointestinal tract, kidney, reproductive organs, skin and mammary glands, bone, brain, heart, lung, thymus, trachea, esophagus, thyroid, adrenals, urinary bladder, lymph nodes and skeletal muscle) are removed and fixed in 10% buffered Zn-Formalin for histological examination. After fixation, the tissues are  
15           processed into paraffin blocks, and 3 mm sections are obtained. All sections are stained with hematoxylin and eosin, and are then subjected to histological analysis.

            The spleen, lymph node, and Peyer's patches of both the transgenic and the control mice are subjected to immunohistology analysis with B cell and T cell  
20           specific antibodies as follows. The formalin fixed paraffin embedded sections are deparaffinized and hydrated in deionized water. The sections are quenched with 3% hydrogen peroxide, blocked with Protein Block (Lipshaw, Pittsburgh, PA), and incubated in rat monoclonal anti-mouse B220 and CD3 (Harlan, Indianapolis, IN). Antibody binding is detected by biotinylated rabbit anti-rat immunoglobulins  
25           and peroxidase conjugated streptavidin (BioGenex, San Ramon, CA) with DAB as a chromagen (BioTek, Santa Barbara, CA). Sections are counterstained with hematoxylin.

            After necropsy, MLN and sections of spleen and thymus from transgenic animals and control littermates are removed. Single cell suspensions are prepared  
30           by gently grinding the tissues with the flat end of a syringe against the bottom of a 100 mm nylon cell strainer (Becton Dickinson, Franklin Lakes, NJ). Cells are

washed twice, counted, and approximately  $1 \times 10^6$  cells from each tissue are then incubated for 10 minutes with 0.5  $\mu\text{g}$  CD16/32(Fc $\gamma$ III/II) Fc block in a 20  $\mu\text{L}$  volume. Samples are then stained for 30 minutes at 2-8°C in a 100  $\mu\text{L}$  volume of PBS (lacking  $\text{Ca}^+$  and  $\text{Mg}^+$ ), 0.1% bovine serum albumin, and 0.01% sodium azide with 0.5  $\mu\text{g}$  antibody of FITC or PE-conjugated monoclonal antibodies against CD90.2 (Thy-1.2), CD45R (B220), CD11b(Mac-1), Gr-1, CD4, or CD8 (PharMingen, San Diego, CA). Following antibody binding, the cells are washed and then analyzed by flow cytometry on a FACScan (Becton Dickinson).

10 Example 9: Functionation of IL-1ra-R Polypeptide

To evaluate whether IL-1ra-R polypeptide affects cellular responses to IL-1, IL-18, or other cytokines, the following experiments were performed. First, murine bone marrow cells were transduced with the IL-1ra-R gene by means of retroviral infection as previously described (Yan *et.al.*, 1999, *Exp. Hematol.* 27:1409-17). Transduced bone marrow was then used to transplant lethally irradiated recipient mice. Upon hematopoietic recovery, bone marrow and spleen cells from IL-1ra-R-transduced and control mice (*i.e.*, mice transduced with empty retroviral vector) were subjected to a series of analyses, including FACS analysis (Figure 12), colony assays (Figures 13A-13B), and  $\gamma$ -interferon production in response to either IL-12 (Figure 14) or IL-12 plus IL-18 (Figure 15).

The data suggest that IL-1ra-R gene expression affects the relative abundance and/or cytokine response of specific subsets of hematopoietic progenitors in the spleen (most prominently G-CSF induced GM-CFC; SCF/Epo induced BFU-E/CFU-Mix; and CSF-1 induced M-CFC) and bone marrow (IL-3 and G-CSF induced GM-CFC; SCF/Epo induced BFU-E/CFU-Mix and IL-3/Epo induced GM-CFC/CFU-Mix).

Expression of IL-1ra-R polypeptide also correlates with decreased surface expression of CD4 and NK1.1 on splenocytes and with a decreased ability of splenocytes to produce  $\gamma$ -interferon in response to IL-12 alone or IL-12 plus IL-18.

While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the invention as  
5 claimed.

## WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

5 (a) the nucleotide sequence as set forth in any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35;

(b) the nucleotide sequence of the DNA insert in ATCC Deposit No. PTA-1423;

10 (c) a nucleotide sequence encoding the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(d) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (c); and

(e) a nucleotide sequence complementary to any of (a) - (c).

15 2. An isolated nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide which is at least about 70 percent identical to the polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, wherein the encoded  
20 polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(b) a nucleotide sequence encoding an allelic variant or splice variant of the nucleotide sequence as set forth in any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, the nucleotide sequence of the DNA insert in  
25 ATCC Deposit No. PTA-1423, or (a);

(c) a region of the nucleotide sequence of any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, the DNA insert in ATCC Deposit No. PTA-1423, (a), or (b) encoding a polypeptide fragment of at least about 25 amino acid residues, wherein the polypeptide fragment has an activity of the  
30 encoded polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or is antigenic;



(d) a region of the nucleotide sequence of any of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 35, the DNA insert in ATCC Deposit No. PTA-1423, or any of (a) - (c) comprising a fragment of at least about 16 nucleotides;

5 (e) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (d); and

(f) a nucleotide sequence complementary to any of (a) - (d).

3. An isolated nucleic acid molecule comprising a nucleotide  
10 sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one conservative amino acid substitution, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ  
15 ID NO: 6, or SEQ ID NO: 36;

(b) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one amino acid insertion, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or  
20 SEQ ID NO: 36;

(c) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one amino acid deletion, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or  
25 SEQ ID NO: 36;

(d) a nucleotide sequence encoding a polypeptide as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 which has a C- and/or N- terminal truncation, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6,  
30 or SEQ ID NO: 36;

(e) a nucleotide sequence encoding a polypeptide as set forth in any of

SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one modification selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the encoded polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(f) a nucleotide sequence of any of (a) - (e) comprising a fragment of at least about 16 nucleotides;

(g) a nucleotide sequence which hybridizes under moderately or highly stringent conditions to the complement of any of (a) - (f); and

(h) a nucleotide sequence complementary to any of (a) - (e).

4. A vector comprising the nucleic acid molecule of any of Claims 1, 2, or 3.

5. A host cell comprising the vector of Claim 4.

6. The host cell of Claim 5 that is a eukaryotic cell.

7. The host cell of Claim 5 that is a prokaryotic cell.

8. A process of producing an IL-1ra-R polypeptide comprising culturing the host cell of Claim 5 under suitable conditions to express the polypeptide, and optionally isolating the polypeptide from the culture.

9. A polypeptide produced by the process of Claim 8.

10. The process of Claim 8, wherein the nucleic acid molecule comprises promoter DNA other than the promoter DNA for the native IL-1ra-R polypeptide operatively linked to the DNA encoding the IL-1ra-R polypeptide.

11. The isolated nucleic acid molecule according to Claim 2, wherein

the percent identity is determined using a computer program selected from the group consisting of GAP, BLASTN, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

- 5           12. A process for determining whether a compound inhibits IL-1ra-R polypeptide activity or IL-1ra-R polypeptide production comprising exposing a cell according to any of Claims 5, 6, or 7 to the compound and measuring IL-1ra-R polypeptide activity or IL-1ra-R polypeptide production in said cell.

- 10           13. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36; and

- (b) the amino acid sequence encoded by the DNA insert in ATCC  
15   Deposit No. PTA-1423.

14. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) an amino acid sequence for an ortholog of any of SEQ ID NO: 2,  
20   SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(b) an amino acid sequence which is at least about 70 percent identical to the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO:  
25   36;

(c) a fragment of the amino acid sequence set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 comprising at least about 25 amino acid residues, wherein the fragment has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or  
30   SEQ ID NO: 36, or is antigenic; and

(d) an amino acid sequence for an allelic variant or splice variant of

the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, the amino acid sequence encoded by the DNA insert in ATCC Deposit No. PTA-1423, (a), or (b).

5           15. An isolated polypeptide comprising the amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one conservative amino acid substitution, wherein the polypeptide has an activity of the polypeptide set  
10 forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(b) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one amino acid insertion, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

15 (c) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one amino acid deletion, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36;

(d) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 which has a C- and/or N- terminal  
20 truncation, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36; and

(e) the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36 with at least one modification  
25 selected from the group consisting of amino acid substitutions, amino acid insertions, amino acid deletions, C-terminal truncation, and N-terminal truncation, wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36.

30           16. An isolated polypeptide encoded by the nucleic acid molecule of any of Claims 1, 2, or 3, wherein the polypeptide has an activity of the

polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36.

17. The isolated polypeptide according to Claim 14, wherein the  
5 percent identity is determined using a computer program selected from the group consisting of GAP, BLASTP, FASTA, BLASTA, BLASTX, BestFit, and the Smith-Waterman algorithm.

18. A selective binding agent or fragment thereof which specifically  
10 binds the polypeptide of any of Claims 13, 14, 15, 55, or 56.

19. The selective binding agent or fragment thereof of Claim 18 that specifically binds the polypeptide comprising the amino acid sequence as set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36, or a  
15 fragment thereof.

20. The selective binding agent of Claim 18 that is an antibody or fragment thereof.

20 21. The selective binding agent of Claim 18 that is a humanized antibody.

22. The selective binding agent of Claim 18 that is a human antibody or fragment thereof.

25

23. The selective binding agent of Claim 18 that is a polyclonal antibody or fragment thereof.

24. The selective binding agent Claim 18 that is a monoclonal antibody  
30 or fragment thereof.

25. The selective binding agent of Claim 18 that is a chimeric antibody or fragment thereof.

26. The selective binding agent of Claim 18 that is a CDR-grafted antibody or fragment thereof.

27. The selective binding agent of Claim 18 that is an antiidiotypic antibody or fragment thereof.

28. The selective binding agent of Claim 18 that is a variable region fragment.

29. The variable region fragment of Claim 28 that is a Fab or a Fab' fragment.

30. A selective binding agent or fragment thereof comprising at least one complementarity determining region with specificity for a polypeptide having the amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36.

31. The selective binding agent of Claim 18 that is bound to a detectable label.

32. The selective binding agent of Claim 18 that antagonizes IL-1ra-R polypeptide biological activity.

33. A method for treating, preventing, or ameliorating an IL-1ra-R polypeptide-related disease, condition, or disorder comprising administering to a patient an effective amount of a selective binding agent according to Claim 18.

34. A selective binding agent produced by immunizing an animal with

a polypeptide comprising an amino acid sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, or SEQ ID NO: 36.

35. A hybridoma which produces a selective binding agent which is  
5 capable of binding a polypeptide according to any of Claims 1, 2, or 3.

36. A method of detecting or quantitating the amount of IL-1ra-R polypeptide using the anti-IL-1ra-R antibody or fragment of Claim 18.

10 37. A composition comprising the polypeptide of any of Claims 13, 14, 15, 55, or 56, and a pharmaceutically acceptable formulation agent.

38. The composition of Claim 37, wherein the pharmaceutically acceptable formulation agent is a carrier, adjuvant, solubilizer, stabilizer, or anti-  
15 oxidant.

39. A polypeptide comprising a derivative of the polypeptide of any of Claims 13, 14, 15, 55, or 56.

20 40. The polypeptide of Claim 39 that is covalently modified with a water-soluble polymer.

41. The polypeptide of Claim 40, wherein the water-soluble polymer is selected from the group consisting of polyethylene glycol, monomethoxy-  
25 polyethylene glycol, dextran, cellulose, poly-(N-vinyl pyrrolidone) polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols, and polyvinyl alcohol.

42. A composition comprising a nucleic acid molecule of any of  
30 Claims 1, 2, or 3 and a pharmaceutically acceptable formulation agent.

43. The composition of Claim 42, wherein said nucleic acid molecule is contained in a viral vector.

44. A viral vector comprising a nucleic acid molecule of any of Claims 1, 2, or 3.

45. A fusion polypeptide comprising the polypeptide of any of Claims 13, 14, 15, 55, or 56 fused to a heterologous amino acid sequence.

46. The fusion polypeptide of Claim 45, wherein the heterologous amino acid sequence is an IgG constant domain or fragment thereof.

47. A method for treating, preventing, or ameliorating a medical condition comprising administering to a patient the polypeptide of any of Claims 13, 14, 15, 55, or 56, or the polypeptide encoded by the nucleic acid of any of Claims 1, 2, or 3.

48. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of any of Claims 13, 14, 15, 55, or 56, or the polypeptide encoded by the nucleic acid molecule of any of Claims 1, 2, or 3 in a sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

49. A device, comprising:

(a) a membrane suitable for implantation; and

(b) cells encapsulated within said membrane, wherein said cells secrete a protein of any of Claims 13, 14, 15, 55, or 56; and  
said membrane is permeable to said protein and impermeable to materials



detrimental to said cells.

50. A method of identifying a compound which binds to an IL-1ra-R polypeptide comprising:

5 (a) contacting the polypeptide of any of Claims 13, 14, 15, 55, or 56 with a compound; and

(b) determining the extent of binding of the IL-1ra-R polypeptide to the compound.

10 51. The method of Claim 50, further comprising determining the activity of the polypeptide when bound to the compound.

52. A method of modulating levels of a polypeptide in an animal comprising administering to the animal the nucleic acid molecule of any of  
15 Claims 1, 2, or 3.

53. A transgenic non-human mammal comprising the nucleic acid molecule of any of Claims 1, 2, or 3.

20 54. A process for determining whether a compound inhibits IL-1ra-R polypeptide activity or IL-1ra-R polypeptide production comprising exposing a transgenic mammal according to Claim 53 to the compound, and measuring IL-1ra-R polypeptide activity or IL-1ra-R polypeptide production in said mammal.

25 55. An isolated polypeptide comprising the amino acid sequence as set forth in either SEQ ID NO: 1 or SEQ ID NO: 3 with at least one amino acid substitution selected from the group consisting of: arginine at position 2; alanine, lysine, or arginine at position 3; serine at position 7; lysine at position 8; alanine, cysteine, lysine, threonine, or serine at position 9; cysteine or phenylalanine at  
30 position 10; arginine or trptophan at position 13; serine at position 15; arginine at position 18; serine or threonine at position 19; threonine at position 21; serine at

position 23; arginine at position 34; tyrosine, serine, or arginine at position 37; lysine, arginine, threonine, or serine at position 38; threonine at position 41; serine, phenylalanine, or alanine at position 43; alanine at position 44; serine or lysine at position 48; alanine, threonine, or phenylalanine at position 52; serine at  
5 position 53; serine at position 54; alanine or tyrosine at position 58; lysine at position 65; phenylalanine at position 66; tyrosine at position 67; serine, tyrosine, or phenylalanine at position 69; lysine or serine at position 73; threonine or arginine at position 78; serine or alanine at position 90; alanine at position 91; serine at position 96; lysine or arginine at position 97; lysine or serine at position  
10 98; alanine at position 100; tyrosine at position 102; arginine or alanine at position 104; lysine at position 105; threonine at position 106; arginine at position 108; lysine, threonine, or trptophan at position 109; threonine or serine at position 110; serine at position 111; serine at position 114; serine at position 116; phenylalanine, cysteine, or tyrosine at position 117; tyrosine at position 121;  
15 serine or alanine at position 123; cysteine, serine, or threonine at position 126; serine at position 136; phenylalanine or arginine at position 138; threonine, arginine, or alanine at position 141; lysine or tyrosine at position 142; trptophan or threonine at position 143; alanine at position 145; threonine or serine at position 147; cysteine at position 151; and serine, cysteine, or phenylalanine at position  
20 152; wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 6.

56. An isolated polypeptide comprising the amino acid sequence as set forth in SEQ ID NO: 5 with at least one amino acid substitution selected from the  
25 group consisting of: arginine at position 21; alanine, lysine, or arginine at position 22; serine at position 26; lysine at position 27; alanine, cysteine, lysine, threonine, or serine at position 28; cysteine or phenylalanine at position 29; arginine or trptophan at position 32; serine at position 34; arginine at position 37; serine or threonine at position 38; threonine at position 40; serine at position 42; arginine at  
30 position 53; tyrosine, serine, or arginine at position 56; lysine, arginine, threonine, or serine at position 57; threonine at position 60; serine, phenylalanine, or alanine

at position 62; alanine at position 63; serine or lysine at position 67; alanine, threonine, or phenylalanine at position 71; serine at position 72; serine at position 73; alanine or tyrosine at position 77; lysine at position 84; phenylalanine at position 85; tyrosine at position 86; serine, tyrosine, or phenylalanine at position 5 88; lysine or serine at position 92; threonine or arginine at position 97; serine or alanine at position 109; alanine at position 110; serine at position 115; lysine or arginine at position 116; lysine or serine at position 117; alanine at position 119; tyrosine at position 121; arginine or alanine at position 123; lysine at position 124; threonine at position 125; arginine at position 127; lysine, threonine, or trptophan 10 at position 128; threonine or serine at position 129; serine at position 130; serine at position 133; serine at position 135; phenylalanine, cysteine, or tyrosine at position 136; tyrosine at position 140; serine or alanine at position 142; cysteine, serine, or threonine at position 145; serine at position 155; phenylalanine or arginine at position 157; threonine, arginine, or alanine at position 160; lysine or 15 tyrosine at position 161; trptophan or threonine at position 162; alanine at position 164; threonine or serine at position 166; cysteine at position 170; and serine, cysteine, or phenylalanine at position 171; wherein the polypeptide has an activity of the polypeptide set forth in any of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 6.

## FIG. 1A

cagggatcag ggttcagga actcaggatc tgcagtgagg accagacacc actgattgca 60  
 gga atg tgt tcc ctc ccc atg gca aga tac tac ata att aaa tat gca 108  
 Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys Tyr Ala  
 1 5 10 15  
 gac cag aag gct cta tac aca aga gat ggc cag ctg ctg gtg gga gat 156  
 Asp Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val Gly Asp  
 20 25 30  
 cct gtt gca gac aac tgc tgt gca gag aag atc tgc aca ctt cct aac 204  
 Pro Val Ala Asp Asn Cys Cys Ala Glu Lys Ile Cys Thr Leu Pro Asn  
 35 40 45  
 aga ggc ttg gac cgc acc aag gtc ccc att ttc ctg ggg atc cag gga 252  
 Arg Gly Leu Asp Arg Thr Lys Val Pro Ile Phe Leu Gly Ile Gln Gly  
 50 55 60  
 ggg agc cgc tgc ctg gca tgt gtg gag aca gaa gag ggg cct tcc cta 300  
 Gly Ser Arg Cys Leu Ala Cys Val Glu Thr Glu Glu Gly Pro Ser Leu  
 65 70 75  
 cag ctg gag gat gtg aac att gag gaa ctg tac aaa ggt ggt gaa gag 348  
 Gln Leu Glu Asp Val Asn Ile Glu Glu Leu Tyr Lys Gly Gly Glu Glu  
 80 85 90 95  
 gcc aca cgc ttc acc ttc ttc cag agc agc tca ggc tcc gcc ttc agg 396  
 Ala Thr Arg Phe Thr Phe Phe Gln Ser Ser Ser Gly Ser Ala Phe Arg  
 100 105 110  
 ctt gag gct gct gcc tgg cct ggc tgg ttc ctg tgt ggc ccg gca gag 444  
 Leu Glu Ala Ala Ala Trp Pro Gly Trp Phe Leu Cys Gly Pro Ala Glu  
 115 120 125  
 ccc cag cag cca gta cag ctc acc aag gag agt gag ccc tca gcc cgt 492  
 Pro Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser Ala Arg  
 130 135 140  
 acc aag ttt tac ttt gaa cag agc tgg tag ggagacagga aactgcgttt 542  
 Thr Lys Phe Tyr Phe Glu Gln Ser Trp  
 145 150  
 tagccttggtg cccccaaacc aagctcatcc tgctcagggt ctatggtagg cagaataatg 602  
 tccccgaaa tatgtccaca tctaataccc aagatctgtg catatgttac catacatgtc 662  
 caaagagggtt ttgcaaatgt gattatgtta aggatcttga aatgaggaga caatcctggg 722  
 ttatccttgt gggctcagtt taatcacaag aaggaggcag gaagggagag tcagagagag 782  
 aatggaagat accatgcttc taattttgaa gatggagtga ggggccttga gccaaacaaat 842  
 gcaggtgttt ttagaagggt gaaaagccaa gggaacggat tctcctctag agtctccgga 902

## FIG. 1B

aggaacacag ctcttgacac atggatttca gctcagtgac acccatttca gacttctgac 962  
ctccacaact ataaaataat aaacttgtgt tattgtaaac ctctaaaaaa aaaaaaaa 1020

## FIG. 2A

cagggatcag ggttccagga actcaggatc tgcagtgagg accagacacc actgattgca 60  
 gga atg tgt tcc ctc ccc atg gca aga tac tac ata att aaa tat gca 108  
     Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys Tyr Ala  
         1                    5                    10                    15  
 gac cag aag gct cta tac aca aga gat ggc cag ctg ctg gtg gga gat 156  
 Asp Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val Gly Asp  
                     20                    25                    30  
 cct gtt gca gac aac tgc tgt gca gag aag atc tgc ata ctt cct aac 204  
 Pro Val Ala Asp Asn Cys Cys Ala Glu Lys Ile Cys Ile Leu Pro Asn  
                     35                    40                    45  
 aga ggc ttg gcc cgc acc aag gtc ccc att ttc ctg ggg atc cag gga 252  
 Arg Gly Leu Ala Arg Thr Lys Val Pro Ile Phe Leu Gly Ile Gln Gly  
             50                    55                    60  
 ggg agc cgc tgc ctg gca tgt gtg gag aca gaa gag ggg cct tcc cta 300  
 Gly Ser Arg Cys Leu Ala Cys Val Glu Thr Glu Glu Gly Pro Ser Leu  
         65                    70                    75  
 cag ctg gag gat gtg aac att gag gaa ctg tac aaa ggt ggt gaa gag 348  
 Gln Leu Glu Asp Val Asn Ile Glu Glu Leu Tyr Lys Gly Gly Glu Glu  
     80                    85                    90                    95  
 gcc aca cgc ttc acc ttc ttc cag agc agc tca ggc tcc gcc ttc agg 396  
 Ala Thr Arg Phe Thr Phe Phe Gln Ser Ser Ser Gly Ser Ala Phe Arg  
                     100                    105                    110  
 ctt gag gct gct gcc tgg cct ggc tgg ttc ctg tgt ggc ccg gca gag 444  
 Leu Glu Ala Ala Ala Trp Pro Gly Trp Phe Leu Cys Gly Pro Ala Glu  
             115                    120                    125  
 ccc cag cag cca gta cag ctc acc aag gag agt gag ccc tca gcc cgt 492  
 Pro Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser Ala Arg  
         130                    135                    140  
 acc aag ttt tac ttt gaa cag agc tgg tag ggagacagga aactgcgttt 542  
 Thr Lys Phe Tyr Phe Glu Gln Ser Trp  
     145                    150  
 tagccttgtg cccccaaacc aagctcatcc tgctcagggt ctatggtagg cagaataatg 602  
 tccccgaaa tatgtccaca tcctaataccc aagatctgtg catatgttac catacatgtc 662  
 caaagagggt ttgcaaatgt gattatgtta aggatcttga aatgaggaga caatcctggg 722  
 ttatccttgt gggctcagtt taatcacaag aaggaggcag gaaggagagag tcagagagag 782  
 aatggaagat accatgcttc taattttgaa gatggagtga ggggccttga gccaaacaaat 842  
 gcagggtgttt ttagaagggt gaaaagccaa gggaacggat tctcctctag agtctccgga 902

## FIG. 2B

aggaacacag ctcttgacac atggatttca gctcagtgac acccatttca gacttctgac 962  
ctccacaact ataaaataat aaacttgtgt tattgtaaac ctctaaaaaa aaaaaaaa 1020

## FIG. 3

gctcccccca ggagaaagga acattctgag gggagtctac accctgtgga gctcaag 57  
 atg gtc ctg agt ggg gcg ctg tgc ttc cgt gag gac cag aca cca ctg 105  
 Met Val Leu Ser Gly Ala Leu Cys Phe Arg Glu Asp Gln Thr Pro Leu  
 1 5 10 15  
 att gca gga atg tgt tcc ctc ccc atg gca aga tac tac ata att aaa 153  
 Ile Ala Gly Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys  
 20 25 30  
 tat gca gac cag aag gct cta tac aca aga gat ggc cag ctg ctg gtg 201  
 Tyr Ala Asp Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val  
 35 40 45  
 gga gat cct gtt gca gac aac tgc tgt gca gag aag atc tgc ata ctt 249  
 Gly Asp Pro Val Ala Asp Asn Cys Cys Ala Glu Lys Ile Cys Ile Leu  
 50 55 60  
 cct aac aga ggc ttg gcc cgc acc aag gtc ccc att ttc ctg ggg atc 297  
 Pro Asn Arg Gly Leu Ala Arg Thr Lys Val Pro Ile Phe Leu Gly Ile  
 65 70 75 80  
 cag gga ggg agc cgc tgc ctg gca tgt gtg gag aca gaa gag ggg cct 345  
 Gln Gly Gly Ser Arg Cys Leu Ala Cys Val Glu Thr Glu Glu Gly Pro  
 85 90 95  
 tcc cta cag ctg gag gat gtg aac att gag gaa ctg tac aaa ggt ggt 393  
 Ser Leu Gln Leu Glu Asp Val Asn Ile Glu Glu Leu Tyr Lys Gly Gly  
 100 105 110  
 gaa gag gcc aca cgc ttc acc ttc ttc cag agc agc tca ggc tcc gcc 441  
 Glu Glu Ala Thr Arg Phe Thr Phe Phe Gln Ser Ser Ser Gly Ser Ala  
 115 120 125  
 ttc agg ctt gag gct gct gcc tgg cct ggc tgg ttc ctg tgt ggc ccg 489  
 Phe Arg Leu Glu Ala Ala Ala Trp Pro Gly Trp Phe Leu Cys Gly Pro  
 130 135 140  
 gca gag ccc cag cag cca gta cag ctc acc aag gag agt gag ccc tca 537  
 Ala Glu Pro Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser  
 145 150 155 160  
 gcc cgt acc aag ttt tac ttt gaa cag agc tgg tag ggagacagga 583  
 Ala Arg Thr Lys Phe Tyr Phe Glu Gln Ser Trp  
 165 170  
 aactgcgttt tagccttgty cccccaaacc aagctcatcc tgctcagggt ctatggtagg 643  
 cagaataatg tccccgaaa tatgtccaca tcctaataccc aagatctgtg catatgttac 703  
 catacatgtc caaagaggtt ttgcaaagt gattatgtta a 744



## FIG. 4A

	1				50
IL-1_alpha	MAEVPKLA	SE	MMAYYS	GNED	DLFFEADGPK QMKCSFQDLD LCPLDGGIQL
IL-1_beta	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
IL-1RA	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
IL-1_delta	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
CS329	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
Tango-77	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
Zilla4	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
IL-1_zeta	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
IL-1RA_beta	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
Spoil_II	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
IL-1_epsilon	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
IL-1_eta	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	51				100
IL-1_alpha	RISDHYSK	G	FRQAAS	VVVA	MDKLRKMLVP CPQTFQENDL STFFPFIFEE
IL-1_beta	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
IL-1RA	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~MEIC RGLRSHLITL
IL-1_delta	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
CS329	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
Tango-77	~~~~~	~~~~~	~~~~~MSFVGENS	GVKMGSEDWE	KDEPQCCELD PAGSPLEPGP
Zilla4	~~~~~	~~~~~	~~~~~MSFVGENS	GVKMGSEDWE	KDEPQCCELD PAGSPLEPGP
IL-1_zeta	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~MSGCDRRETE
IL-1RA_beta	~~~~~	~~~~~	~~~~~MRGTPGDADG	GGRAVYQS..	.....
Spoil_II	~~~~~	~~~~~	~~~~~MRGTPGDADG	GGRAVYQSSE	SNAVGMGLWR LRPSALTLS
IL-1_epsilon	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
IL-1_eta	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	101				150
IL-1_alpha	EPIFFDTWDN	E	EAYVHD	APVR	SLNCTLRDSQ QKSLVMSGPY ELKALHLQGG
IL-1_beta	~~~~~	~~~~~	~~~~~	~~~~~APVR	SLNCTLRDSQ QKSLVMSGPY ELKALHLQGG
IL-1RA	LLFLFHSETI	C	RP	SGRKSSK	IQAFRIWDVN QKTFYLRNN. QLVAGYLQGP
IL-1_delta	~~~~~	~~~~~	~~~~~	~~~~~MVL	SG ALCFRMKDSA LKVLYLHNN. QLLAGGLHAG
CS329	~~~~~	~~~~~	~~~~~	~~~~~MCSLPM	ARYYIIKYAD QKALYTRDG. QLLVGDPVAD
Tango-77	SLPTMNFVH.	.	T.....	.....	.....
Zilla4	SLPTMNFVH.	.	TSPKVKNLN	PKKFSIHDQD	HKVLVLDSG. NLIA..VPDK
IL-1_zeta	TKGKNSFKKR	L	RGP	KVKNLN	PKKFSIHDQD HKVLVLDSG. NLIA..VPDK
IL-1RA_beta	.....	.....	MCK	PITGTINDLN	QQVWTLQGG. NLVA..VPRS
Spoil_II	VEAPAFSAPL	C	TL	PFPPVCK	PITGTINDLN QQVWTLQGG. NLVA..VPRS
IL-1_epsilon	~~~~~	~~~~~	~~~~~	~~~~~MEKALKIDT	PQQGSIQDIN HRVWVLQDQ. TLIA..VPRK
IL-1_eta	~~~~~	~~~~~	~~~~~	~~~~~MNPQREAA	PKSYAIRDSR QMVWVLSGN. SLIA..APLS
	151				200
IL-1_alpha	DMEQQVVFSM	...	SFVQ	GEE	SNDKIPVALG LKEKNLYLSC VLKDDK..PT
IL-1_beta	DMEQQVVFSM	...	SFVQ	GEE	SNDKIPVALG LKEKNLYLSC VLKDDK..PT
IL-1RA	NVNLEEKIDV	V	P.....	IEP	....HALFLG IHGGMCLSC VKSGDE..TR
IL-1_delta	KVIKGEEISV	V	PNRWLDASL	....SPVILG	VQGSQCLSC .GVGQE..PT
CS329	NC.CAEKICT	L	PNRGLDRTK	....VPIFLG	IQGSRCLAC VETEEG..PS
Tango-77	....KIFFA	L	ASSLSSA.S	AEKGSPIILG	VSKGEFCLYC DKDKGQSHPS
Zilla4	NYIRPEIFFA	L	ASSLSSA.S	AEKGSPIILG	VSKGEFCLYC DKDKGQSHPS
IL-1_zeta	NYIRPEIFFA	L	ASSLSSA.S	AEKGSPIILG	VSKGEFCLYC DKDKGQSHPS
IL-1RA_beta	DSVTPVTAV	I	TCKYPEALE	QGRGDPIYLG	IQNPMECLYC EKVGEQ..PT
Spoil_II	DSVTPVTAV	I	TCKYPEALE	QGRGDPIYLG	IQNPMECLYC EKVGEQ..PT
IL-1_epsilon	DRMSPVTIAL	I	SCRHVETLE	KDRGNPIYLG	LNGLNLCLMC AKVGDQ..PT
IL-1_eta	RSIKPVTLHL	I	ACRDTEFSD	KEKGNMYYLG	IKGDLCLFC AEIQGK..PT

## FIG. 4B

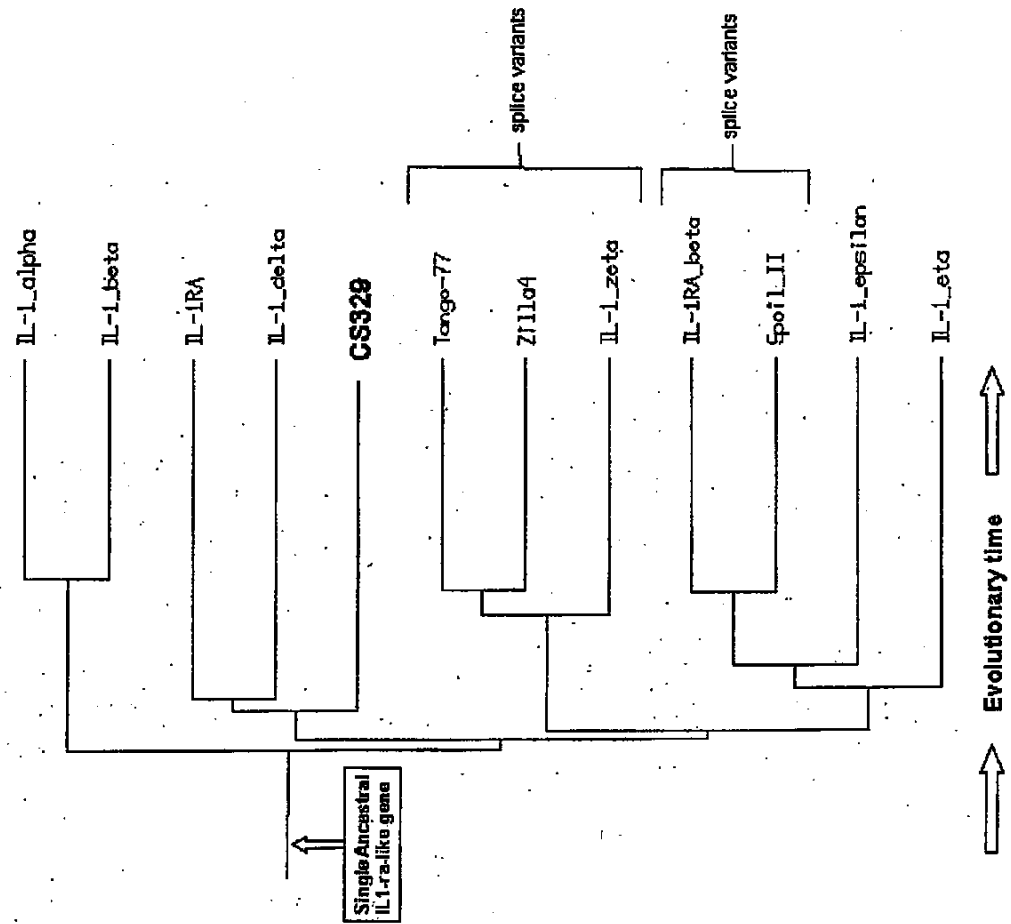
	201		250
IL-1_alpha	LQLESVDPKN Y..PKKKMEK RFVFNKIEIN NKLEFESAQF PNWYISTSQA		
IL-1_beta	LQLESVDPKN Y..PKKKMEK RFVFNKIEIN NKLEFESAQF PNWYISTSQA		
IL-1RA	LQLEAVNITD LSEN RKQDKR .FAFIRSDSG PTTSFESAAC PGWFLCTAME		
IL-1_delta	LTLEPVNIME LYLGAKEKSKS .FTFYRRDMG LTSSFESAAY PGWFLCTVPE		
CS329	LQLEDVNIEE LYKGGEETR .FTFFQSSSG SAFRLEAAAW PGWFLCGPAE		
Tango-77	LQLKKEKLMK LAAQKESARR PFIFYRAQVG SWNMLESAAH PGWFICTSCN		
Zilla4	LQLKKEKLMK LAAQKESARR PFIFYRAQVG SWNMLESAAH PGWFICTSCN		
IL-1_zeta	LQLKKEKLMK LAAQKESARR PFIFYRAQVG SWNMLESAAH PGWFICTSCN		
IL-1RA_beta	LQLKEQKIMD LYGQPEPV.K PFLFYRAKTG RTSTLESVAF PDWFIA.SSK		
Spoil_II	LQLKEQKIMD LYGQPEPV.K PFLFYRAKTG RTSTLESVAF PDWFIA.SSK		
IL-1_epsilon	LQLKEKDIND LYNQPEPV.K SFLFYHSQSG RNSTFESVAF PGWFIASSE		
IL-1_eta	LQLKEKNIMD LYVEKKAQ.K PFLFFHNKEG STSVFQSVSY PGWFIATSTT		

	251		290
IL-1_alpha	ENMPVFL... .GGTKGGQDI TDFTMQFVSS ~~~~~		
IL-1_beta	ENMPVFL... .GGTKGGQDI TDFTMQFVSS ~~~~~		
IL-1RA	ADQPVSLTNM PDEG...VMV TKFYFQEDE~ ~~~~~		
IL-1_delta	ADQPVRLTQL PENGGWNAPI TDFYFQQCD~ ~~~~~		
CS329	PQOPVQLTKE SEPSAR.... TKFYFEQSW~ ~~~~~		
Tango-77	CNEPVGVTDK FENRKH.... IEFSFQPVCK AEMSPSEVSD		
Zilla4	CNEPVGVTDK FENRKH.... IEFSFQPVCK AEMSPSEVSD		
IL-1_zeta	CNEPVGVTDK FENRKH.... IEFSFQPVCK AEMSPSEVSD		
IL-1RA_beta	RDQPIILTSE LGKSYN.... TAFELNIND~ ~~~~~		
Spoil_II	RDQPIILTSE LGKSYN.... TAFELNIND~ ~~~~~		
IL-1_epsilon	GGCPLILTQE LGKANT.... TDFGLTMLF~ ~~~~~		
IL-1_eta	SGQPIFLTKE RGITNN.... TNFYLDSE~ ~~~~~		

# Phylogenetic Tree of the Emerging IL1-ra Gene Family

FIG. 5



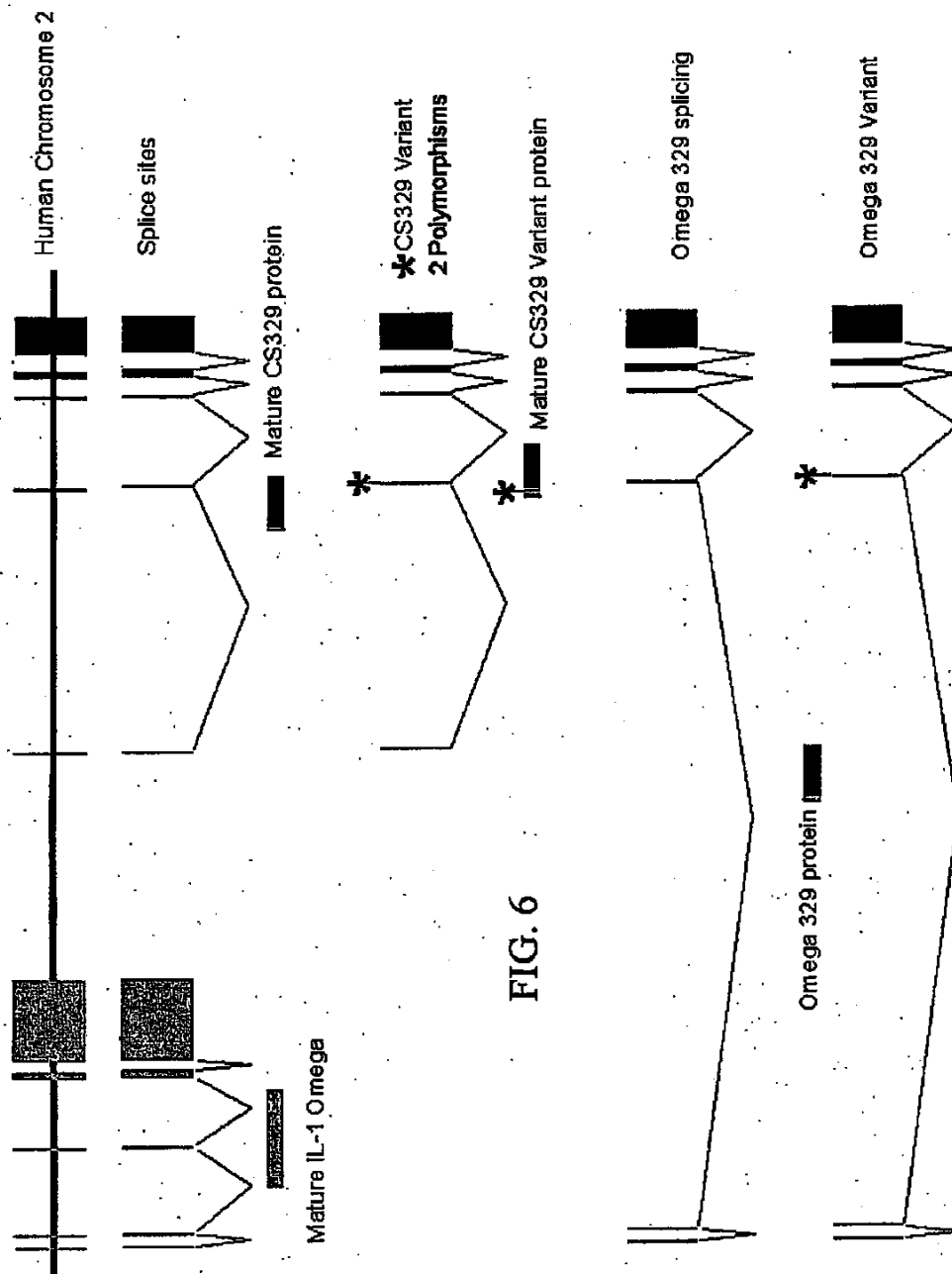


FIG. 6

Juxtaposition of IL-1 Omega and CS329 exons results in a hybrid protein that harbors a potential functional secretion signal sequence

## FIG. 7

atg tgc tcc ctt ccc atg gca aga tac tac ata atc aag gat gca cat	48
Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys Asp Ala His	
1 5 10 15	
caa aag gct ttg tac aca cgg aat ggc cag ctc ctg ctg gga gac cct	96
Gln Lys Ala Leu Tyr Thr Arg Asn Gly Gln Leu Leu Leu Gly Asp Pro	
20 25 30	
gat tca gac aat tat agt cca gag aag gtc tgt atc ctt cct aac cga	144
Asp Ser Asp Asn Tyr Ser Pro Glu Lys Val Cys Ile Leu Pro Asn Arg	
35 40 45	
ggc cta gac cgc tcc aag gtc ccc atc ttc ctg ggg atg cag gga gga	192
Gly Leu Asp Arg Ser Lys Val Pro Ile Phe Leu Gly Met Gln Gly Gly	
50 55 60	
agt tgc tgc ctg gcg tgt gta aag aca aga gag gga cct ctc ctg cag	240
Ser Cys Cys Leu Ala Cys Val Lys Thr Arg Glu Gly Pro Leu Leu Gln	
65 70 75 80	
ctg gag gat gtg aac atc gag gac cta tac aag gga ggt gaa caa acc	288
Leu Glu Asp Val Asn Ile Glu Asp Leu Tyr Lys Gly Gly Glu Gln Thr	
85 90 95	
acc cgt ttc acc ttt ttc cag aga agc ttg gga tct gcc ttc agg ctt	336
Thr Arg Phe Thr Phe Phe Gln Arg Ser Leu Gly Ser Ala Phe Arg Leu	
100 105 110	
gag gct gct gcc tgc cct ggc tgg ttt ctc tgt ggc cca gct gag ccc	384
Glu Ala Ala Ala Cys Pro Gly Trp Phe Leu Cys Gly Pro Ala Glu Pro	
115 120 125	
cag cag cca gtg cag ctc acc aaa gag agt gaa ccc tcc acc cat act	432
Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser Thr His Thr	
130 135 140	
gaa ttc tac ttt gag atg agt cgg taa	459
Glu Phe Tyr Phe Glu Met Ser Arg	
145 150	



## FIG. 9A

actagtctcc catagacaac agctgaatgt acgagggtcag aagcaaggcc tgccccagaa 60  
 ccattgcaag ccagggtgctg tcttgattgt agcctcataa aaaactgatg cagaattgcc 120  
 ccaccaacat gctccagatt cctgctccac agaaaccctg tgaactaacc atgttgcttt 180  
 tagattctgc agtaagttga taatctgcag taaataacat tcgatgaaag agaaacatgt 240  
 gtagttactt tattatgac aaaactttat ttctccactc ttccattttt ctttctcaga 300  
 attgacacca gccttttact aacccaaata gcctatttaa atgctgatca tacttctctt 360  
 gttaaactgtt acctgttccc aaaagggtaca attccctttc gaccatagct gcatctccca 420  
 cctgcacacc aggatgtttc tcatatttct acctaaaaca ttgggggacta caagtgaag 480  
 caaaagaggg ggtccatctc agaaccctcag gtatttagct gtaaaactca cttgtcaggc 540  
 cagcttgaca ggtttacagt ttgtagaagg accagaaaga aggtagccaa gacagaagag 600  
 gcaacctctg cttgtcctag aaccttcagt ccatatacat ctaagctccc cagcaccatt 660  
 tctaccacag acctctcaga gttcctgagg atgcagaccc caggacactg acctcagttt 720  
 ccaggcaggg tttctgcaca ccccttcac actgcctgac tgggagttag tctcatgggtg 780  
 caacactact ttgggacact gtacccatcc cctcgaccta cagaaaccat tcacttttca 840  
 aggtcacctc ctataggaag tatttgaaaa gatgagagtc atgggtcattt gctatgataa 900  
 tattctgtgc ttatctccct gtaaaaagtt ggcttgggggt ctctggcatg catctgacct 960  
 taaggttgga gctgcaccaa tatgttttta agcaccggc ataatgcttc gcaaaatttc 1020  
 agaacatggg ttgtacagaa tgtactttcc tccactcata caaacccttg taaaagagta 1080  
 gtttgaatcc caactcattc ttgaaggcca cctttttagt ggtgacagaa tttaaaaata 1140  
 cagaatttaa aaatacttta tcccaggga gtcacactt ctaaattccag aatgaaagaa 1200  
 gaaatagaaa cacacttggt gtggcggtgg tgggtggtgat ggtggtcgtg gtggtggtgg 1260  
 tgggtggtgg ggtgatgggt gtggtggtgg tgggtggtgg ggtcgtggtg gtgtaatgat 1320  
 cacagtaaag tgaggcatca tggcctgaga ggtcaggca tcacagctat tcaagtgaag 1380  
 actacctact actgatttta gagttctata attttagtag cagccacagg cctggggcct 1440  
 gggcctatat ttccagagag gaaatgttca cagcaggtca actgcagaca gtgaagatca 1500  
 gaaatgtttc ataactcagg catcagagaa aaggcaaagg agctgatgga ctttatcctg 1560  
 aaaaagcaaa atccaaccca cctcatgctt aatgcattca aaggctctgc ggacagaagaa 1620

## FIG. 9B

tacattttgc tttttattat tataaattac ctggagaata tttttgtctg aattatctcc 1680  
caaataattaa ccataaaaat aaaaaattcc atgtgtgctt ctcccagggg ctataaagcc 1740  
cctggtctta gagttgttgg ggcaaaacct gacctttgaa gtagttactt ttgaagatgc 1800  
cataccatac atttggccac ttggagagag tctaattgtca catctaaagg gttactctga 1860  
tgctctgttt tctcatatgc ccttggctta cagctaacta tggctccagc taaactataa 1920  
agttcccttg caacagagat ggtacgctat gtgtctttga cacagcagaa taaatgctta 1980  
gtgaacatta ctgattgcct gacaggacac ctcacacttt ggtactttca acagagggat 2040  
gtaaacttat gaagaacaat gaagaatgaa tattggcaat aaaagcaaaa attgggttaac 2100  
ccaattctag ctctgaaatc attttttaggt agtgggaagt ctttttgttt tgttttattca 2160  
ctttacatcc caattgctgt cctccctcca agttccccac caccaccaca gtcctttttc 2220  
cctcccttc tctctgaga gaatggagaa cctcctgga tattccccca tcatgaaaca 2280  
ttaagtctct gcagggctag acacttcccc cagtgaggcc agtcagggca gccagctag 2340  
aaaaagcata tcccacagac agacaacagc ttttgggata gccccgttc agttgttttag 2400  
gatccacatg aaggctgagc tgcacatctg ctacatatga atgaggaggc ctaggtccag 2460  
cctgtgtatg ttcttttggtt ggtggttcag actctgagag cccaaggggt ccaggtcagt 2520  
tgactctgtt ggtcttctg tggacaccct gtcccttcc agcccacaat ccttccccta 2580  
atccttctcc ttctcacttc cataagagtg tgaggagtct ttaaaaacat gaagcatttt 2640  
atctccccag ggcaacacat ggaaatgaaa gattgtgaaa agtaatttaa agaaaaagaa 2700  
aaaaaaattt aacaaggaat aagaatcttg tttctctgaa aatgcttaag agtgtggaaa 2760  
acataaactg gattctaata gaatgcaatt ggattgtaat gaaaacctat caaagttatg 2820  
aaatagcttt cactacctg cacaaaatct cttggcatgt gtgttggttg caaattttct 2880  
tgttagttaa aaaccacaac aataacaaca aaatagcaaa aattgggtct cagcctcatt 2940  
cattttttct catttcttgc tctgtgatcg tctgggtctt aagctgacac ctcaccaatt 3000  
cctcatcaag acctttgtgg aaatttgcaa atgtcccaaa aaggagaatt acaataagtc 3060  
agagaacgtt ctgtccaatt ctttatccct agtgatggat gagtaaagga tgtataagag 3120  
atggataaat ggactgatgt acagataaat gaaggaatat gtacatggtt aggtggatag 3180  
atgacttact caacagatga gtagaaggat gagaaataga tggacagctg gactgaggca 3240



## FIG. 9C

tgcaaagtca actggagaac tgagtctctt gaccatgcac tgtccaggggt ctcatattcc 3300  
 ctagagtcca gggcccatgg ctctgtgcc atcccatgc aaatctaagg ttaatacggtt 3360  
 ctacagctga gtttccttac atatgtgtct cagtaagttt gtatcaacta attaaatctg 3420  
 aaaggagttc cttctgatct tcccaaacag agccacactc gtgatgaagt cagccctgct 3480  
 tcattgtggt tctctggatg catctggctt ccatcagcat aatctttcta ttcttgatcc 3540  
 ttccaacctc ttcaggctc agacagaacc ccatggagca tcaaagaggt ttgaccccag 3600  
 cattgtttat gtagctgcaa aaccactaat aacacagtca atgacagtag ctacagagac 3660  
 agcaggtcag tgtctggcct ctgtcaaggc tttatgagtg actctctccc cttcccgcaa 3720  
 atactcatta atctcccac ctcttatta tttggactgt gttgaagata ttatgaaatc 3780  
 tctgggctct tcttccgga tctagagcca attacagatt ctgtaggttt gacccaccct 3840  
 gaccagacat tataaacaca gtgctggtgc cctgaagaaa acagttggag actccaggca 3900  
 ttagaatcca ggcaccagga actacaggtc agtggtgaca gtcggtctct ctgtgtatct 3960  
 cttacacaca cacacataca cacacacaac acaacataca cacacataca acacacaaca 4020  
 catacacata caacacatac acacacacaa cacttttctg taatgtctcc aaaattctca 4080  
 ggctctaggg aagaagaaat gtcttttaga gaatgcggtg tgatgttcta taagtctagg 4140  
 aatacttgat agaatttaat gagaagtata gattaggtca aagcaagggt actacatatt 4200  
 tggaaccaca gagttttgaa agtcatctca aaagaaatta ttaggccag agatgttcaa 4260  
 aaaatgtttt gtttgtgaca tatggaagct cccatggaga cattctgtga ttctcatcaa 4320  
 tagacagtag ggatgccacc aagggtgctaa cgtcttcac accccatcat ctatcataca 4380  
 tccaaatggt ttctttgaaa acaatctcct tgtgaaactt aaagtagcct tgaaaatata 4440  
 ataatcttgt ccagcctctc atttcaatgg gaatagattg aaggcctaag gacaaaaaca 4500  
 aaaaacaaaa caaacaaaat aaaaacacca aaaaaaaaaac ccataaaatg aatgagtagc 4560  
 taagttattt ttagaatcca gcctttcagt caaagcttga ttcatgcata tctgtgttct 4620  
 gatcttaagg tgctgtgtct gtcagttgta tagttggata gaggtacaga tgagctatat 4680  
 acatcatgct tcaagatttc aggatcttat aacttttata aagcaaataa tttgtcttaa 4740  
 tgcacactaa taaacaatat agcaaagttt gacaggagtt cagagtactg ttagagaagt 4800  
 gaagggaaga attttggtat gatagtaaag gggaaaatca aattttgagt catggaatca 4860

## FIG. 9D

tacatagttt gacatagaaa gaaccttggc aaccacataa tctaatacat gagcccaaga 4920  
 actggcctgt gtttttaaga tctcattctc agctgttatg taactgaaca gacaagatac 4980  
 taagcccaag tatagtgaag ccatgtccag tgatcttaat aggagtgaca ggaatggttg 5040  
 gtgatgaaga ggggtggatt ttgagcagga ataccaaaag caatgctgac tgtgcccttg 5100  
 gagagaatta gcatgagtcc ttgagagaaa aatgagatgc tattgcacaa gcaacctagg 5160  
 gccagatggg gtcaagatag gtggccatcg tggactttag aaccaggcag gaatgtgatc 5220  
 agagatgtac tttatgtagg ttaggtttga ttcagaaacc aggaggggta gcatgtttac 5280  
 aatggtgact aaaaacaagc acaagggttat actttaaaaga aataatctct gaaaagaagg 5340  
 gaggtatatt ttcagtgcg gaaagaggaa tattacaaaa gtgagaggag tagatttgag 5400  
 aaagagaagt ggattgtgga ggagcagatg ctcaccacgc ccttactc acttgaactg 5460  
 acacccaaag atgaagggtg gctgtggact gctgaagctc agcctgtggc tgggaagcag 5520  
 taaacaaaat tgctcatcac agctgtacaa gatattccat agcatataaa aataaaagtg 5580  
 cttaggctat tctcttacia ctctcagcct tatgaatgac ccggaaggaa aagaactcta 5640  
 caatgtgcct gtgtctgttc ttacttcctc tgccacaagc aaaagagcct tgggaatttg 5700  
 ctcagagggg acgtcatcaa acaggctggc cttgaggctg ggctgttatt cgtctacctg 5760  
 ggatagagga attcgctatt cttttataat ccaagtgtgg cctggggacc agcagcatta 5820  
 ttaagacctg gttgcatgtt tgaaatgcag tctcagattt catcccagac ctaaagagta 5880  
 aactgtttt catgaggata caagattaag aaatatgcat tagagagtaa ttggctaaat 5940  
 gggtaaagt catgcaagca ggaggatctg attgactccc caggaccac acagtccca 6000  
 tgccgtagag cacatctgta atcacagtag gcgtatgatg aaatgggagg tgaatcaaga 6060  
 gaatctctag cagctacggg ctggccagcc tcccatgcac agcactaaat aaggcaagga 6120  
 ccaataacctg aagttgtccc attaccttca catatacacc acggcatgtg tgtacttgta 6180  
 ctcacacata caaacaata cacacgtgca cacatacaaa actcagagat taaggacaat 6240  
 tggcctgaca tatcagttcc taagcctggc tcattgcttg taacactaca agcagtatta 6300  
 aataaggata ggcgagagaa cagttaccga atggttcaga agtggggcca tgctgtgac 6360  
 tttaaacaaa tgtttcatat ttttaataa taacacttag attacaaaat aaatttacta 6420  
 caggaaaatg ttaagaacta tcaacaacca ttgactatcc tgtcgccac aaatgagtg 6480

## FIG. 9E

tataacaagc accagccgtc cttgtccaca tgtgtgtgtg tctacacagc tatgaattta 6540  
attgggataa taatgtgcac attctttacg gcctgcagtt tttacttcat gtatttgaaa 6600  
tgtttgtgcc acaaattgtca tctttaagga gcatatcctt atttcctgga tttatcattc 6660  
cctttcagcc gactggacat tgacagcatt tccaactttt caaccttgta aaaataacta 6720  
attgaactat tttataacta agcatttggg caatcaatta cctctgcctg gaatgggggc 6780  
aacaacacat gcaatcatgg gaaagccagg atgctgctgt ctgatcccta gccctggcat 6840  
tcgtgcagaa cctcactctc atctgtgccc tgatatecct cactctcaag tcttttccca 6900  
gtgactttta aaggcaacag aatcatatag ccaataatga aagctacttg gtctacagtt 6960  
gtgtggcggt ttttatagat attttcttca tttacatttc aaatgctatc ccaaaagtcc 7020  
cctataccct cccccaccct gctcccctac ccactcactc ccacttcttg gccctggcctt 7080  
tcccccttac tggggcatat aaagtgtgct agaccaaggg gcctctcttc ccaatgatgg 7140  
ccaactaggc cattttctgc tacatatgca gctagagaca ccagttctgg ggttactggt 7200  
tagttcatat tgttgttcta cctatgggggt tgcagacccc ttcagctctt gactactttc 7260  
tctagctcct ccattgggag cctgtgttcc catcctatag atgactgtga gcatccactt 7320  
ctgtatttgc caggcactgg catatgaaat agtatctgca tttggtggct gattatggga 7380  
tggacccccg ggtggggcag tctctggatg gtccatcctt tcatcttagc tccaaacttt 7440  
gtctctgcaa cttcttccat ggatatttta gtccctaata tagggagaaa tgaagtatcc 7500  
acaagttgat cttccttctt gattttctta tgttttagaa gttgtatctt ggatatttcta 7560  
ggtttctggg ctaatatcca cttatcagtg agtacatata aagtgaattc ttttgtgatt 7620  
aggttacctc actcaagatg atattctcca ctatgttcat agcagcccta tttatagtag 7680  
ccagaagctg gaaagaaccc agtccctcaa cagaggaatg gatacagaaa atgtggcaca 7740  
tttatgcaat ggagtaccac tcagatatta aaaacaacga atttatgaaa ttctcgggca 7800  
aaaccctatc taaagaccag gaataaggaa aagatggact gcctgcctgc agctggggaga 7860  
gctgggggaga cctttgtgga ttctgttaata cttaggggta cggaacagct tgtggctgga 7920  
taattctgag ctccagcatg tctgcccccc aaaaaacatt ctgtttttct gaaagccttt 7980  
ttcttctttg cctcagtga gaccagacac tccaactgc agga atg tgc tcc ctt 8036  
ccc atg gca aga tac tac at gtaagtaa tcttaacgat cgctcaatca 8084

## FIG. 9F

aggggcctgg agatcacatg agaagggaaa aggctgagtc aaagggacaa agctccctct 8144  
 agccacagaa atctcaaaca ctgaataatt gatcttcacg tttgtcaatc acaacagccc 8204  
 tctttcctgg tgacagaatg gaacaactgt aagagtggta ttgcttagtc cattttacag 8264  
 acccggaaac tcaacctcca cgagggtata caattttcct catgtcatgc aattacccaa 8324  
 aagcagagag tgggatcgga ctctctgttc tctaaactga tgtagctagt tcttagaaag 8384  
 ctcaaacaat cttgagtccc aaggacagca cctttatggt cacctggatt gataacctata 8444  
 tcaaaaaaaaa aaaaaggctc cactagatag ccctggctac cctgaaactc tcaactgtgta 8504  
 catttaggtg accacgaact cacagagatc tgccttccaa gtgctgggat taaagtatgt 8564  
 accaccacac ctgcatcttt gacaataact gagtggatc taaattcttc cagtggctaa 8624  
 acagttaagt. ccagttccc aaagtctgag aaaaatgccg ggtggtgaaa tctgtacaga 8684  
 cctttgttct taatgtacaa gtgagcctgc tttaaaaaca atacgcaagc tgtttttgct 8744  
 attgctaagt gttgcagaga cagaaaaggc tcccagaagt ggtaactttg gtccagaggt 8804  
 tctgtttctca aactcattgt gagctctgaa agcaactgat gggcagctct gaaatcagct 8864  
 gggcaattag gctaataaca ggcataattt taatgtttca cacgcatgac agttcctccc 8924  
 cagctgcct agtacatact taccctccta ggcacgtcat tagaccata ggtataacca 8984  
 gtgactaatc aggccttggc ctaattctaa gttggcctcc tatataagtg ccaactcagag 9044  
 tgtacctcat catggctgta gtgggcccag agtctagga catagacttt tctattgtcc 9104  
 aatttctgat ttgtgaattt tctacaaaaa gaattttttt taattttaca aatcaaatca 9164  
 cagttactac atcttcagtt ccttcattaa ttagtggtac tatttaaaaa aataaaataa 9224  
 atcaagctca gaaacatcat ggataggggt cattgtatct ccagggtagc tgagcttcaa 9284  
 agcaactcct cagacagcca tgaaaacatc ctcaattacc tcatgagaag aactattgt 9344  
 catttctgga gcctctgata atcctgagcc taggcagctt tgggatgaaa caatttctac 9404  
 ccttattgga acagtgtccc tctcctgtct ggaaacaatt caccaaaggc tccatgtggt 9464  
 tgtccagtaa ggtggtatgg ggacagaaat ggacaatgat ccctgagggc agtgatccat 9524  
 taaccttgcc ctctatttc ag a atc aaq gat gca cat caa aaq gct ttg tac 9577  
aca cgg aat gcc cag ctg ctg gga gac cct gat tca gac aat tat 9625  
agt cca g gtgatcttc cgggtggtggg ggtgggggag tggaggggag ggtgtggggg 9681

## FIG. 9G

gggctctctt ccagaagttg cttagtgtcc atctgccaca aggccttgat tctttccttc 9741  
 aattgtgtct ctagagacat gagaatattg tcacagtgat aaggagaaga ggtaggggca 9801  
 gtttcttcct gtaaaaaatg aattccattt accctgcagt ctccatacag aaacaggcca 9861  
 gaggggggca gaccagtaa cttctagctg agccctacct tgcttaaaac ctgccatctg 9921  
 tggccccctc actgtctgaa ttgcattctg tcttacctcc cag ag aag qtc tgt atc 9978  
ctt cct aac cga qgc cta qac cgc tcc aag qtc ccc atc ttc ctg qgg 10026  
atg caq qga qga agt tgc tgc ctg gcg tgt qta aag aca aga qag qga 10074  
cct ctc ctg caq ctg gag gtgagacacc cctcctcatt gcagtcagta 10122  
 ctgccactgg aacatagtga catctttgaa cccacatgtc ccctctcttg tttcccatct 10182  
 atctctcttt gcctccagct gagggactct agcctttggg gatgtacaga aagaacatgg 10242  
 cttcgaaaaa ctcttcccta ttgagtcctt ctttggccaa gcctctgagg cactaagggc 10302  
 tgacgtccca accaaacact catttcatct cacagctgtc tccctttccc cacag gat 10360  
gtg aac atc gag qac cta tac aag qga qgt qaa caa acc acc cgt ttc 10408  
acc ttt ttc caq aga agc ttg qga tct gcc ttc agg ctt gag gct gct 10456  
gcc tgc cct qgc tgg ttt ctc tgt qgc cca gct gag ccc cag cag cca 10504  
gtg caq ctc acc aaa gag agt qaa ccc tcc acc cat act qaa ttc tac 10552  
ttt gag atg agt cgg taa agagacataa ggctggggcc tcgtctagt 10600  
 cccccagttt gagatcttct tgctcagcat ctctggaaag cagaataagg aagataccaa 10660  
 agatgtttgg gtcttaatcc ccagaatctg tgaccgtgtt acattaaatg gcaaagggat 10720  
 ttttttttct cttcatggtc catttgggccc cattggaatc atctgaggcc tcatgaggag 10780  
 aaggaagagg tcagagggag actggggcaa actttggtac taaaagtaac aatggagaca 10840  
 gggaccataa gctgatgggt aacagtgggt tctagaaacc ggaaatgatg agagctctcc 10900  
 tgacacaggt tctggatttt tctggactga agaattggtga aataatacag ctccattatt 10960  
 ttaagccact gagtttgaga tcattcaatg aagctgtcat aataaaacct gtgcttcaca 11020  
 tacaattcaa tattggtagg cccccgggtg atttcttggg aagacatcta gggattctcc 11080  
 tggatgctga ttccagggtc cagtggagtc cctgggttga agagatttca caaccagaa 11140  
 catcaggctc gactcttcta aaagtcgctc gttgcacccc ttgcctgaga gcattagcaa 11200

## FIG. 9H

ttctatttc ataggaaatc tgtgtccctg cccctgctaa agcagggagc ctggaccgtc 11260  
 ctgatttagt gagggtgag ctgctggcac tttttgtgt caccagtgtc ttaagcagt 11320  
 atggagcaca aaagatcttt actgagaaga tggccatgaa gctctggcta gacaccaaga 11380  
 atatgatata agcagagcta cagcacaaga tgagccaatg aggaaagcca ttcagggagg 11440  
 ctaagcccag cttcccaaag ggacagctaa cctggactc aaatgaatag gggttttcct 11500  
 ggcagagAAC ataggtcaag cattctaggt agaatcagca attcagaaag gtgtgagaga 11560  
 ggcattggaga gctccaggca tgtctgggt atggtgtgtc attcttgtgg caagaatcca 11620  
 acgtctgtgg ttaaggagtt gctgaaaatt aaaataggaa aatgggtaga gtctaattgt 11680  
 gaatgacttg caaaggagtt tagcccataa gtggggagct cagaggagtc atctaaggat 11740  
 tgcaagcagg ggccctgtga tcattgctgg accagcctag gtgctacaga gcctaccttc 11800  
 agctctgcat cctcactcac atccaggtac cttcagaggt caatttctgt gctctgggttc 11860  
 tatgggtagc ctgacctgt ttcattcttct tgtataactt aggcacataa gcttagggac 11920  
 tggtagagtt tacttgagt attggtgaat caggcagcac caaactacaa gttgttcagg 11980  
 gctttaccaa gggggcactg attggagaat tggaatgagg gtggttagaa tgcattcaga 12040  
 aaacaagggg aagaaaaatt tgattgctta aagtggaaag tcccaactta aatgttagtc 12100  
 agtagtttct aattacttga gtctctaatt agaggttagt tggcagtttc tggttagtta 12160  
 atctaagttt cattttctta ggctatgacc attctctgag tcgcatgtta gcaatgcagt 12220  
 aagaactcaa gaccagaat agcctctgtt aattatttta gcaatgatca ctcatttctg 12280  
 ttgcctcta ttgagatctg ttcccatgga ccaccaggc acatcaggcc tcctagtacc 12340  
 aacataataa tgattgctgc acagacaaaa tatttttttt cagtatctgg tatttgctac 12400  
 atttccatta gtgctggagg gaaggctaca acgaccatga aggcattggc cctgccttct 12460  
 aaggacttac aatgtaatag gagccctgac attataaagt gggtcacctt gtttcaaact 12520  
 gagccaaact gaggctgagg gcttagatta gtggtaggtc actttccaga catgttcagt 12580  
 gctaagaaaa acacattctg gggttagtta gatgttttag ttcatttgat aagaagccca 12640  
 atgattggac tttcaacttc tggaacccat gtggtggaag agagaaccaa cttctgacca 12700  
 tttgggtcat ggcacatccc ctaccatcac aagaactcac caaaataaat tagaaaaatc 12760  
 aagaaaaact catatcctat agacctctgg tagaattagc agaacgtgc tgtggcactt 12820

## FIG. 9I

gggatttgaa actcaaaaat ggaagaagct acttgtgacc gttcaagact ccaggaggagc 12880  
tcctctgaca catcccacga ctcaggetta aattccttct tctccctaga aggccacgcc 12940  
atcttctcaa ccaggccaca gatgctataa ttatgtaaat gtgtgggaga ggcacacttt 13000  
agatcttatc cactagt 13017

FIG. 10C

Spleen  
Lymph node  
Thymus  
Peripheral blood Leukocyte  
Bone marrow  
Fetal liver

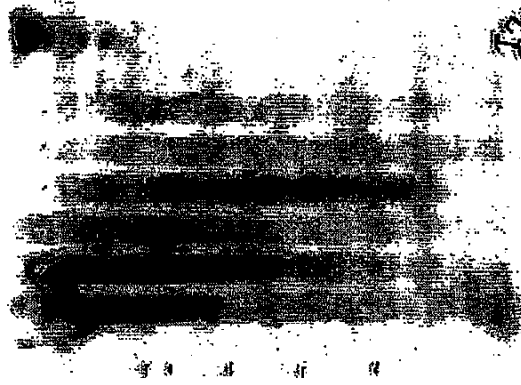


FIG. 10B

Heart  
Brain  
Placenta  
Lung  
Liver  
Skeletal muscle  
Kidney  
Pancreas

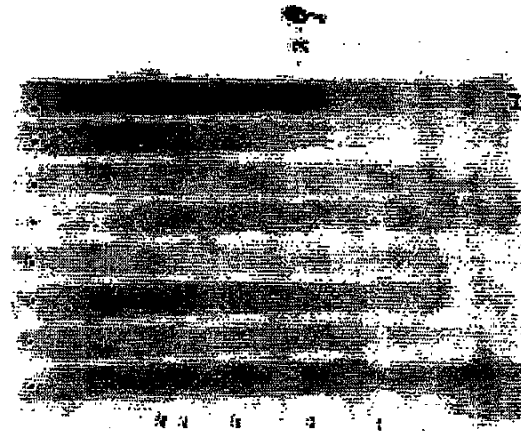


FIG. 10A

Heart  
Brain  
Spleen  
Lung  
Liver  
Skeletal muscle  
Kidney  
Testis

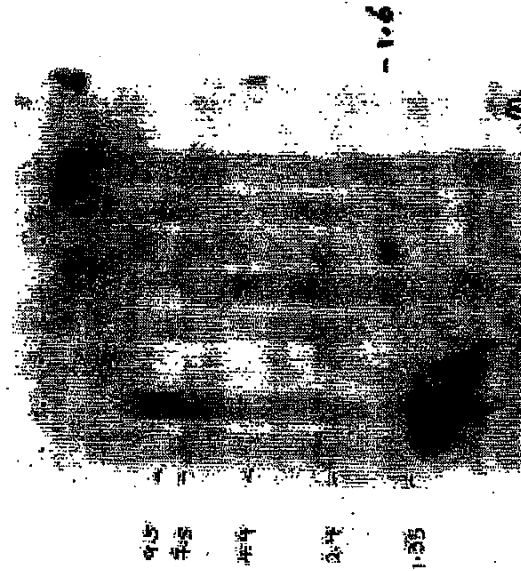




FIG. 11A

FIG. 11B

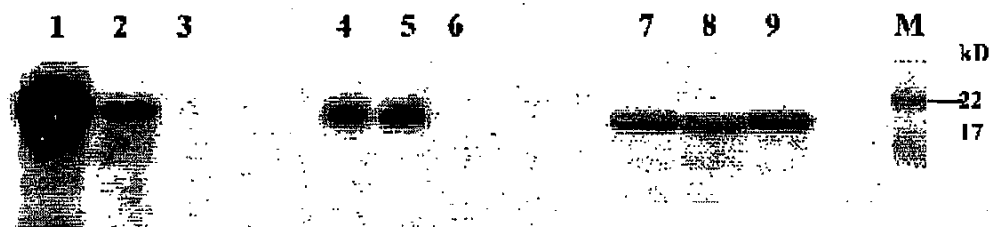


FIG. 12

**Spleen**

	CD4+	CD8+	CD4+ CD8+	CD3+	NK1.1+	CD3+ NK1.1+
control mice	20.18	3.72	1.67	24.07	3.06	1.4
CS329 mice	15.89	3.99	0.37	22.9	2.08	1.1
Difference:	-4.29	0.27	-1.3	-1.17	-0.98	-0.3

**Bone Marrow**

	CD4+	CD8+	CD4+ CD8+	CD3+	NK1.1+	CD3+ / NK1.1+
control mice	2.62	2.54	0.49	3.88	1.26	0.49
CS329 mice	2.46	2.35	0.41	4.42	1.53	0.57
Difference:	0.16	0.19	0.08	0.54	0.27	0.08

FIG. 13A

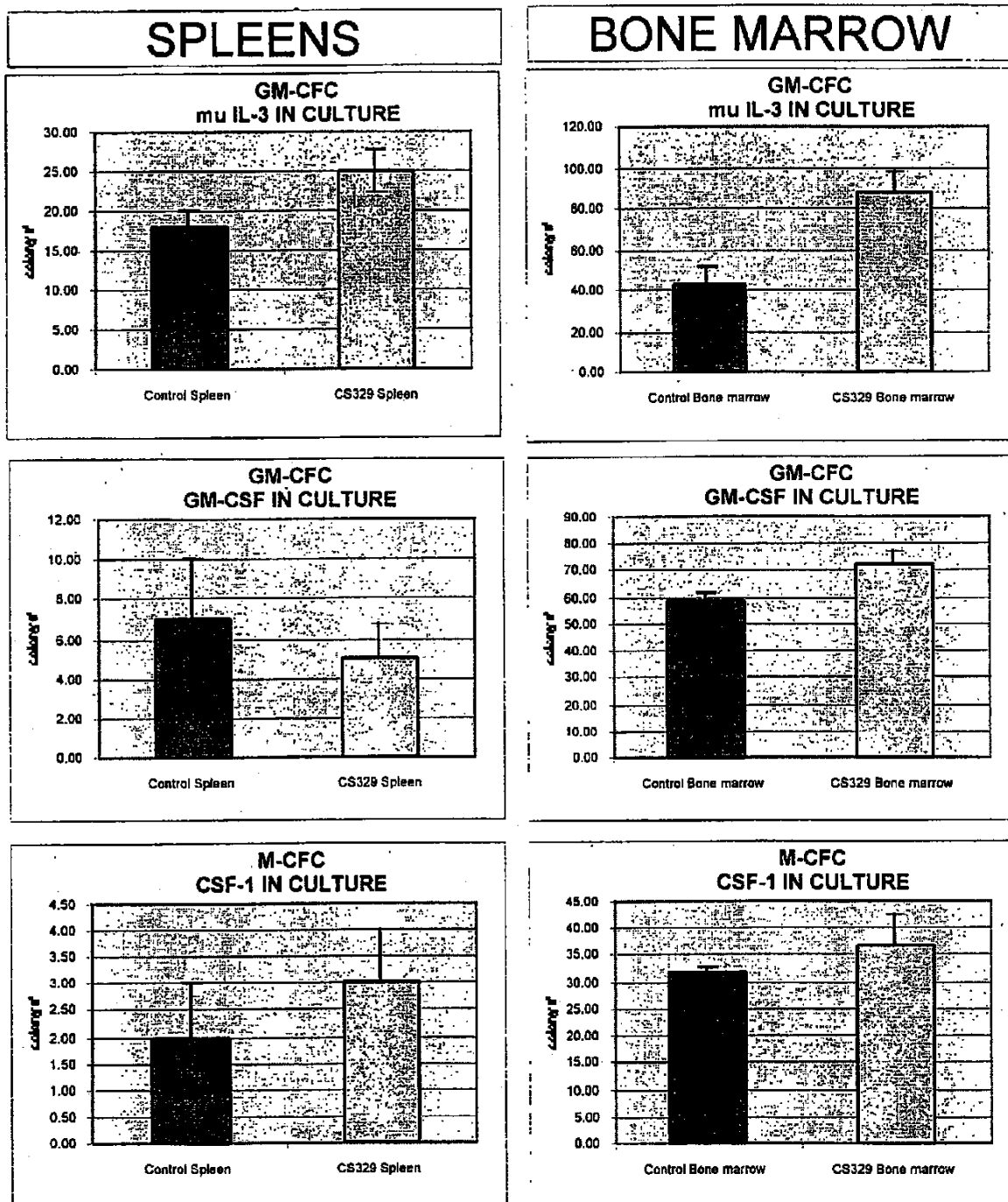


FIG. 13B

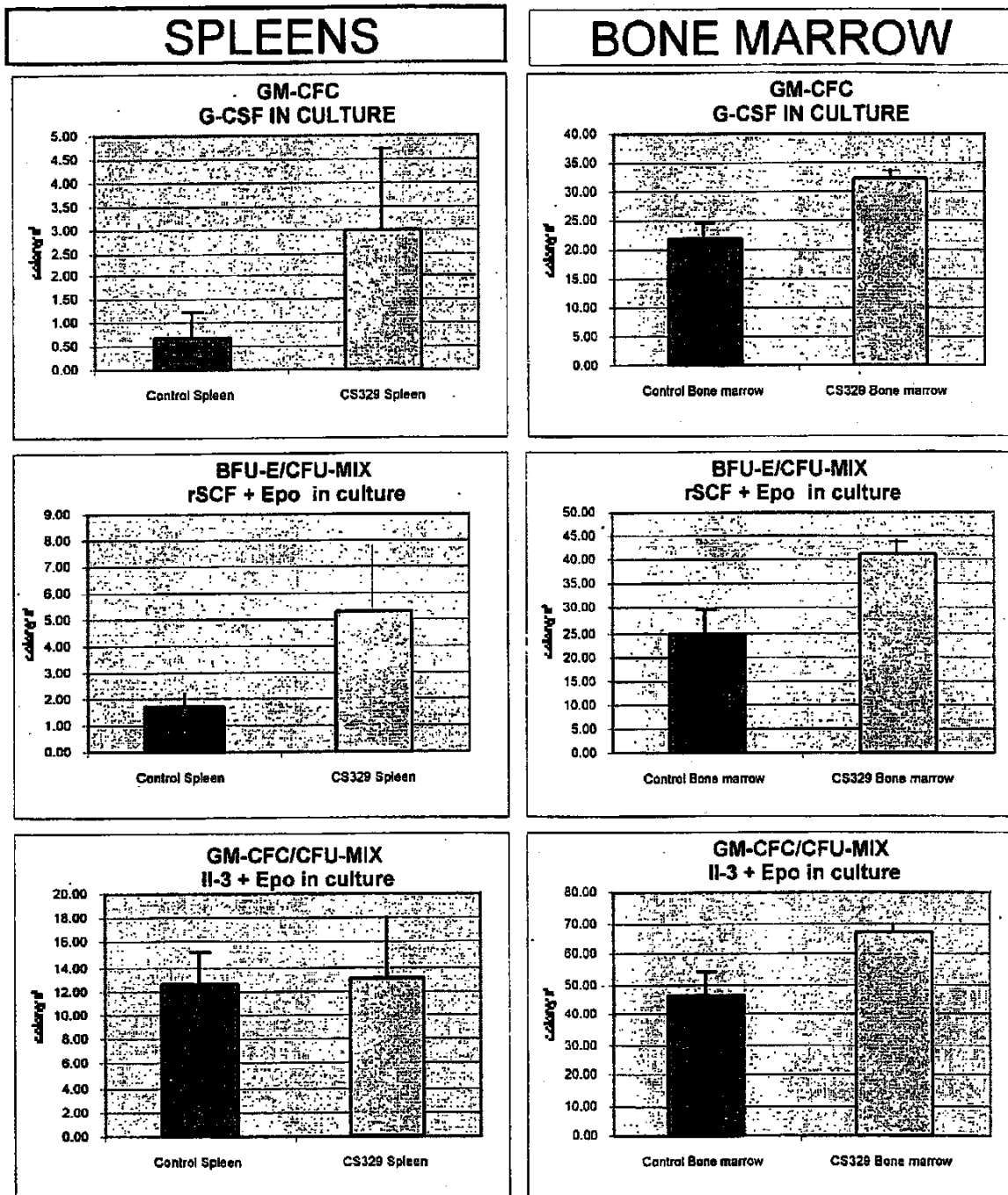


FIG. 14

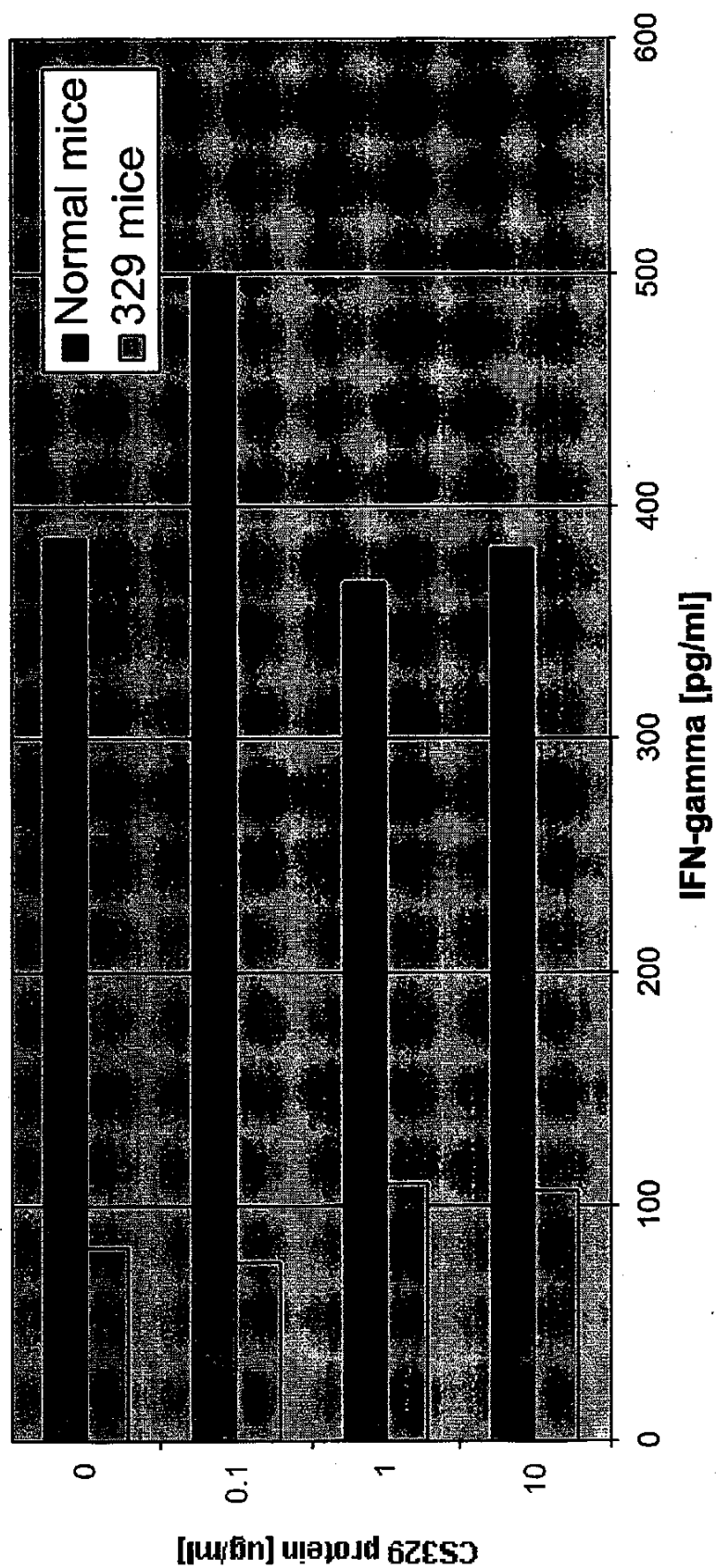
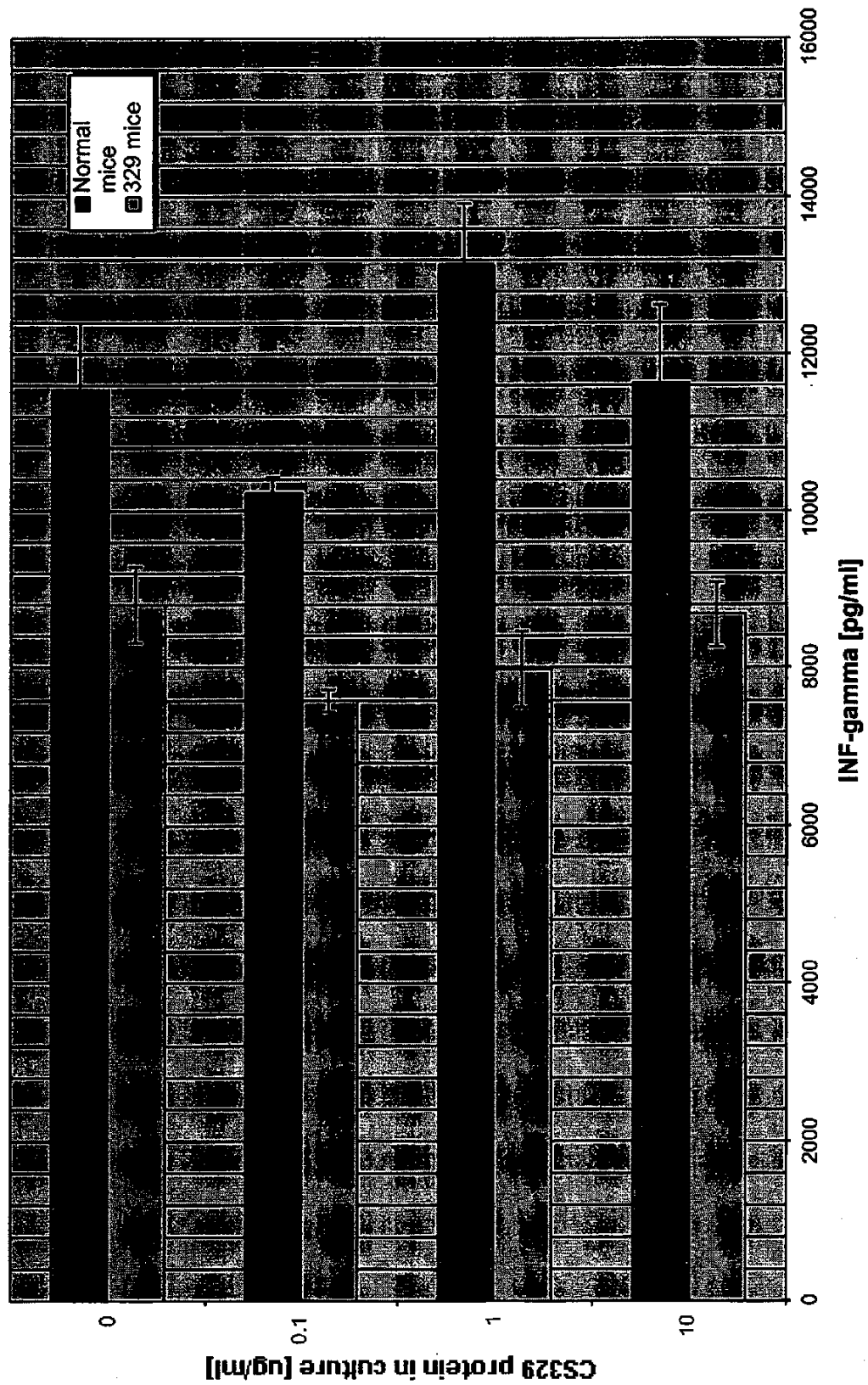


FIG. 15



## SEQUENCE LISTING

<110> Saris, Christiaan M.  
 Giles, Jennifer  
 Mu, Sharon X.  
 Xia, Min  
 Bass, Michael B.  
 Craveiro, Roger

<120> Interleukin-1 Receptor Antagonist-Related Molecules and  
 Uses Thereof

<130> 00-1213

<140>

<141>

<150> 60/170,191

<151> 1999-12-10

<150> 60/188,053

<151> 2000-03-09

<150> 60/194,521

<151> 2000-04-04

<150> 60/195,910

<151> 2000-04-10

<160> 37

<170> PatentIn Ver. 2.0

<210> 1

<211> 1020

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (64)..(522)

<400> 1

```

cagggatcag ggttcagga actcaggatc tgcagtgagg accagacacc actgattgca 60

gga atg tgt tcc ctc ccc atg gca aga tac tac ata att aaa tat gca 108
  Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys Tyr Ala
    1             5             10             15

gac cag aag gct cta tac aca aga gat ggc cag ctg ctg gtg gga gat 156
  Asp Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val Gly Asp
    20             25             30

cct gtt gca gac aac tgc tgt gca gag aag atc tgc aca ctt cct aac 204
  Pro Val Ala Asp Asn Cys Cys Ala Glu Lys Ile Cys Thr Leu Pro Asn
    35             40             45

aga ggc ttg gac cgc acc aag gtc ccc att ttc ctg ggg atc cag gga 252
  Arg Gly Leu Asp Arg Thr Lys Val Pro Ile Phe Leu Gly Ile Gln Gly
    50             55             60

```

ggg agc cgc tgc ctg gca tgt gtg gag aca gaa gag ggg cct tcc cta 300  
 Gly Ser Arg Cys Leu Ala Cys Val Glu Thr Glu Gly Pro Ser Leu  
 65 70 75

cag ctg gag gat gtg aac att gag gaa ctg tac aaa ggt ggt gaa gag 348  
 Gln Leu Glu Asp Val Asn Ile Glu Glu Leu Tyr Lys Gly Gly Glu Glu  
 80 85 90 95

gcc aca cgc ttc acc ttc ttc cag agc agc tca ggc tcc gcc ttc agg 396  
 Ala Thr Arg Phe Thr Phe Phe Gln Ser Ser Ser Gly Ser Ala Phe Arg  
 100 105 110

ctt gag gct gct gcc tgg cct ggc tgg ttc ctg tgt ggc ccg gca gag 444  
 Leu Glu Ala Ala Ala Trp Pro Gly Trp Phe Leu Cys Gly Pro Ala Glu  
 115 120 125

ccc cag cag cca gta cag ctg acc aag gag agt gag ccc tca gcc cgt 492  
 Pro Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser Ala Arg  
 130 135 140

acc aag ttt tac ttt gaa cag agc tgg tag ggagacagga aactgcgttt 542  
 Thr Lys Phe Tyr Phe Glu Gln Ser Trp  
 145 150

tagccttggtg cccccaaacc aagctcatcc tgctcagggt ctatggtagg cagaataatg 602

tcccccgaaa tatgtccaca tcctaattccc aagatctgtg catatgttac catacatgtc 662

caaagagggtt ttgcaaatgt gattatgtta aggatcttga aatgaggaga caatcctggg 722

ttatccttgt gggctcagtt taatcacaag aaggaggcag gaaggagagag tcagagagag 782

aatggaagat accatgcttc taattttgaa gatggagtga ggggccttga gccacaaat 842

gcagggtgttt ttagaagggtg gaaaagccaa gggaacggat tctcctctag agtctccgga 902

aggaacacag ctcttgacac atggatttca gctcagtgac acccatttca gacttctgac 962

ctccacaact ataaaataat aaacttgtgt tattgtaaac ctctaaaaaa aaaaaaaa 1020

<210> 2  
 <211> 152  
 <212> PRT  
 <213> Homo sapiens

<400> 2  
 Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys Tyr Ala Asp  
 1 5 10 15  
 Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val Gly Asp Pro  
 20 25 30  
 Val Ala Asp Asn Cys Cys Ala Glu Lys Ile Cys Thr Leu Pro Asn Arg  
 35 40 45  
 Gly Leu Asp Arg Thr Lys Val Pro Ile Phe Leu Gly Ile Gln Gly Gly  
 50 55 60  
 Ser Arg Cys Leu Ala Cys Val Glu Thr Glu Glu Gly Pro Ser Leu Gln  
 65 70 75 80



Leu Glu Asp Val Asn Ile Glu Glu Leu Tyr Lys Gly Gly Glu Glu Ala  
                             85                            90                            95  
 Thr Arg Phe Thr Phe Phe Gln Ser Ser Ser Gly Ser Ala Phe Arg Leu  
                             100                            105                            110  
 Glu Ala Ala Ala Trp Pro Gly Trp Phe Leu Cys Gly Pro Ala Glu Pro  
                             115                            120                            125  
 Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser Ala Arg Thr  
                             130                            135                            140  
 Lys Phe Tyr Phe Glu Gln Ser Trp  
 145                            150

<210> 3  
 <211> 1020  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (64)..(522)

<400> 3  
 cagggatcag ggttcagga actcaggatc tgcagtgagg accagacacc actgattgca 60  
  
 gga atg tgt tcc ctc ccc atg gca aga tac tac ata att aaa tat gca 108  
   Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys Tyr Ala  
      1                            5                            10                            15  
  
 gac cag aag gct cta tac aca aga gat ggc cag ctg ctg gtg gga gat 156  
   Asp Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val Gly Asp  
                             20                            25                            30  
  
 cct gtt gca gac aac tgc tgt gca gag aag atc tgc ata ctt cct aac 204  
   Pro Val Ala Asp Asn Cys Cys Ala Glu Lys Ile Cys Ile Leu Pro Asn  
                             35                            40                            45  
  
 aga ggc ttg gcc cgc acc aag gtc ccc att ttc ctg ggg atc cag gga 252  
   Arg Gly Leu Ala Arg Thr Lys Val Pro Ile Phe Leu Gly Ile Gln Gly  
                             50                            55                            60  
  
 ggg agc cgc tgc ctg gca tgt gtg gag aca gaa gag ggg cct tcc cta 300  
   Gly Ser Arg Cys Leu Ala Cys Val Glu Thr Glu Glu Gly Pro Ser Leu  
      65                            70                            75  
  
 cag ctg gag gat gtg aac att gag gaa ctg tac aaa ggt ggt gaa gag 348  
   Gln Leu Glu Asp Val Asn Ile Glu Glu Leu Tyr Lys Gly Gly Glu Glu  
      80                            85                            90                            95  
  
 gcc aca cgc ttc acc ttc ttc cag agc agc tca ggc tcc gcc ttc agg 396  
   Ala Thr Arg Phe Thr Phe Phe Gln Ser Ser Ser Gly Ser Ala Phe Arg  
                             100                            105                            110  
  
 ctt gag gct gct gcc tgg cct ggc tgg ttc ctg tgt ggc ccg gca gag 444  
   Leu Glu Ala Ala Ala Trp Pro Gly Trp Phe Leu Cys Gly Pro Ala Glu  
                             115                            120                            125

ccc cag cag cca gta cag ctc acc aag gag agt gag ccc tca gcc cgt 492  
 Pro Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser Ala Arg  
           130                          135                          140

acc aag ttt tac ttt gaa cag agc tgg tag ggagacagga aactgcgttt 542  
 Thr Lys Phe Tyr Phe Glu Gln Ser Trp  
           145                          150

tagccttgtg cccccaaacc aagctcatcc tgctcagggt ctatggtagg cagaataatg 602

tccccgaaa tatgtccaca tcctaataccc aagatctgtg catatgttac catacatgtc 662

caaagagggt ttgcaaatgt gattatgtta aggatcttga aatgaggaga caatcctggg 722

ttatccttgt gggctcagtt taatcacaag aaggaggcag gaagggagag tcagagagag 782

aatggaagat accatgcttc taattttgaa gatggagtga ggggccttga gccaacaaat 842

gcagggtgttt ttagaagggtg gaaaagccaa gggaacggat tctcctctag agtctccgga 902

aggaacacag ctcttgacac atggatttca gctcagtgac acccatttca gacttctgac 962

ctccacaact ataaaataat aaacttgtgt tattgtaaac ctctaaaaaa aaaaaaaaa 1020

<210> 4

<211> 152

<212> PRT

<213> Homo sapiens

<400> 4

Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys Tyr Ala Asp  
           1                          5                          10                          15

Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val Gly Asp Pro  
                           20                          25                          30

Val Ala Asp Asn Cys Cys Ala Glu Lys Ile Cys Ile Leu Pro Asn Arg  
           35                          40                          45

Gly Leu Ala Arg Thr Lys Val Pro Ile Phe Leu Gly Ile Gln Gly Gly  
           50                          55                          60

Ser Arg Cys Leu Ala Cys Val Glu Thr Glu Glu Gly Pro Ser Leu Gln  
           65                          70                          75                          80

Leu Glu Asp Val Asn Ile Glu Glu Leu Tyr Lys Gly Gly Glu Glu Ala  
                           85                          90                          95

Thr Arg Phe Thr Phe Phe Gln Ser Ser Ser Gly Ser Ala Phe Arg Leu  
           100                          105                          110

Glu Ala Ala Ala Trp Pro Gly Trp Phe Leu Cys Gly Pro Ala Glu Pro  
           115                          120                          125

Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser Ala Arg Thr  
           130                          135                          140

Lys Phe Tyr Phe Glu Gln Ser Trp  
           145                          150

<210> 5  
 <211> 744  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> CDS  
 <222> (58)..(573)

<400> 5  
 gctccccgcca ggagaaagga acattctgag gggagtctac accctgtgga gctcaag 57

atg gtc ctg agt ggg gcg ctg tgc ttc cgt gag gac cag aca cca ctg 105  
 Met Val Leu Ser Gly Ala Leu Cys Phe Arg Glu Asp Gln Thr Pro Leu  
 1 5 10 15

att gca gga atg tgt tcc ctc ccc atg gca aga tac tac ata att aaa 153  
 Ile Ala Gly Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys  
 20 25 30

tat gca gac cag aag gct cta tac aca aga gat ggc cag ctg ctg gtg 201  
 Tyr Ala Asp Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val  
 35 40 45

gga gat cct gtt gca gac aac tgc tgt gca gag aag atc tgc ata ctt 249  
 Gly Asp Pro Val Ala Asp Asn Cys Cys Ala Glu Lys Ile Cys Ile Leu  
 50 55 60

cct aac aga ggc ttg gcc cgc acc aag gtc ccc att ttc ctg ggg atc 297  
 Pro Asn Arg Gly Leu Ala Arg Thr Lys Val Pro Ile Phe Leu Gly Ile  
 65 70 75 80

cag gga ggg agc cgc tgc ctg gca tgt gtg gag aca gaa gag ggg cct 345  
 Gln Gly Gly Ser Arg Cys Leu Ala Cys Val Glu Thr Glu Glu Gly Pro  
 85 90 95

tcc cta cag ctg gag gat gtg aac att gag gaa ctg tac aaa ggt ggt 393  
 Ser Leu Gln Leu Glu Asp Val Asn Ile Glu Glu Leu Tyr Lys Gly Gly  
 100 105 110

gaa gag gcc aca cgc ttc acc ttc ttc cag agc agc tca ggc tcc gcc 441  
 Glu Glu Ala Thr Arg Phe Thr Phe Phe Gln Ser Ser Ser Gly Ser Ala  
 115 120 125

ttc agg ctt gag gct gct gcc tgg cct ggc tgg ttc ctg tgt ggc ccg 489  
 Phe Arg Leu Glu Ala Ala Ala Trp Pro Gly Trp Phe Leu Cys Gly Pro  
 130 135 140

gca gag ccc cag cag cca gta cag ctc acc aag gag agt gag ccc tca 537  
 Ala Glu Pro Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser  
 145 150 155 160

gcc cgt acc aag ttt tac ttt gaa cag agc tgg tag ggagacagga 583  
 Ala Arg Thr Lys Phe Tyr Phe Glu Gln Ser Trp  
 165 170

aactgcgttt tagccttggt cccccaacc aagctcatcc tgctcagggt ctatggtagg 643

cagaataatg tcccccgaaa tatgtccaca tcctaattccc aagatctgtg catatgttac 703

catacatgtc caaagaggtt ttgcaaattgt gattatgtta a

744

&lt;210&gt; 6

&lt;211&gt; 171

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 6

Met Val Leu Ser Gly Ala Leu Cys Phe Arg Glu Asp Gln Thr Pro Leu  
 1 5 10 15

Ile Ala Gly Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys  
 20 25 30

Tyr Ala Asp Gln Lys Ala Leu Tyr Thr Arg Asp Gly Gln Leu Leu Val  
 35 40 45

Gly Asp Pro Val Ala Asp Asn Cys Cys Ala Glu Lys Ile Cys Ile Leu  
 50 55 60

Pro Asn Arg Gly Leu Ala Arg Thr Lys Val Pro Ile Phe Leu Gly Ile  
 65 70 75 80

Gln Gly Gly Ser Arg Cys Leu Ala Cys Val Glu Thr Glu Glu Gly Pro  
 85 90 95

Ser Leu Gln Leu Glu Asp Val Asn Ile Glu Glu Leu Tyr Lys Gly Gly  
 100 105 110

Glu Glu Ala Thr Arg Phe Thr Phe Phe Gln Ser Ser Ser Gly Ser Ala  
 115 120 125

Phe Arg Leu Glu Ala Ala Ala Trp Pro Gly Trp Phe Leu Cys Gly Pro  
 130 135 140

Ala Glu Pro Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser  
 145 150 155 160

Ala Arg Thr Lys Phe Tyr Phe Glu Gln Ser Trp  
 165 170

&lt;210&gt; 7

&lt;211&gt; 269

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 7

Met Ala Glu Val Pro Lys Leu Ala Ser Glu Met Met Ala Tyr Tyr Ser  
 1 5 10 15

Gly Asn Glu Asp Asp Leu Phe Phe Glu Ala Asp Gly Pro Lys Gln Met  
 20 25 30

Lys Cys Ser Phe Gln Asp Leu Asp Leu Cys Pro Leu Asp Gly Gly Ile  
 35 40 45

Gln Leu Arg Ile Ser Asp His His Tyr Ser Lys Gly Phe Arg Gln Ala  
 50 55 60

Ala Ser Val Val Val Ala Met Asp Lys Leu Arg Lys Met Leu Val Pro  
 65 70 75 80  
 Cys Pro Gln Thr Phe Gln Glu Asn Asp Leu Ser Thr Phe Phe Pro Phe  
 85 90 95  
 Ile Phe Glu Glu Glu Pro Ile Phe Phe Asp Thr Trp Asp Asn Glu Ala  
 100 105 110  
 Tyr Val His Asp Ala Pro Val Arg Ser Leu Asn Cys Thr Leu Arg Asp  
 115 120 125  
 Ser Gln Gln Lys Ser Leu Val Met Ser Gly Pro Tyr Glu Leu Lys Ala  
 130 135 140  
 Leu His Leu Gln Gly Gln Asp Met Glu Gln Gln Val Val Phe Ser Met  
 145 150 155 160  
 Ser Phe Val Gln Gly Glu Glu Ser Asn Asp Lys Ile Pro Val Ala Leu  
 165 170 175  
 Gly Leu Lys Glu Lys Asn Leu Tyr Leu Ser Cys Val Leu Lys Asp Asp  
 180 185 190  
 Lys Pro Thr Leu Gln Leu Glu Ser Val Asp Pro Lys Asn Tyr Pro Lys  
 195 200 205  
 Lys Lys Met Glu Lys Arg Phe Val Phe Asn Lys Ile Glu Ile Asn Asn  
 210 215 220  
 Lys Leu Glu Phe Glu Ser Ala Gln Phe Pro Asn Trp Tyr Ile Ser Thr  
 225 230 235 240  
 Ser Gln Ala Glu Asn Met Pro Val Phe Leu Gly Gly Thr Lys Gly Gly  
 245 250 255  
 Gln Asp Ile Thr Asp Phe Thr Met Gln Phe Val Ser Ser  
 260 265

&lt;210&gt; 8

&lt;211&gt; 153

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 8

Ala Pro Val Arg Ser Leu Asn Cys Thr Leu Arg Asp Ser Gln Gln Lys  
 1 5 10 15  
 Ser Leu Val Met Ser Gly Pro Tyr Glu Leu Lys Ala Leu His Leu Gln  
 20 25 30  
 Gly Gln Asp Met Glu Gln Gln Val Val Phe Ser Met Ser Phe Val Gln  
 35 40 45  
 Gly Glu Glu Ser Asn Asp Lys Ile Pro Val Ala Leu Gly Leu Lys Glu  
 50 55 60  
 Lys Asn Leu Tyr Leu Ser Cys Val Leu Lys Asp Asp Lys Pro Thr Leu  
 65 70 75 80

Gln Leu Glu Ser Val Asp Pro Lys Asn Tyr Pro Lys Lys Lys Met Glu  
                                     85                                    90                                    95

Lys Arg Phe Val Phe Asn Lys Ile Glu Ile Asn Asn Lys Leu Glu Phe  
                                     100                                    105                                    110

Glu Ser Ala Gln Phe Pro Asn Trp Tyr Ile Ser Thr Ser Gln Ala Glu  
                                     115                                    120                                    125

Asn Met Pro Val Phe Leu Gly Gly Thr Lys Gly Gly Gln Asp Ile Thr  
                                     130                                    135                                    140

Asp Phe Thr Met Gln Phe Val Ser Ser  
 145                                    150

&lt;210&gt; 9

&lt;211&gt; 177

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 9

Met Glu Ile Cys Arg Gly Leu Arg Ser His Leu Ile Thr Leu Leu Leu  
   1                                    5                                    10                                    15

Phe Leu Phe His Ser Glu Thr Ile Cys Arg Pro Ser Gly Arg Lys Ser  
                                     20                                    25                                    30

Ser Lys Ile Gln Ala Phe Arg Ile Trp Asp Val Asn Gln Lys Thr Phe  
                                     35                                    40                                    45

Tyr Leu Arg Asn Asn Gln Leu Val Ala Gly Tyr Leu Gln Gly Pro Asn  
                                     50                                    55                                    60

Val Asn Leu Glu Glu Lys Ile Asp Val Val Pro Ile Glu Pro His Ala  
   65                                    70                                    75                                    80

Leu Phe Leu Gly Ile His Gly Gly Lys Met Cys Leu Ser Cys Val Lys  
                                     85                                    90                                    95

Ser Gly Asp Glu Thr Arg Leu Gln Leu Glu Ala Val Asn Ile Thr Asp  
                                     100                                    105                                    110

Leu Ser Glu Asn Arg Lys Gln Asp Lys Arg Phe Ala Phe Ile Arg Ser  
                                     115                                    120                                    125

Asp Ser Gly Pro Thr Thr Ser Phe Glu Ser Ala Ala Cys Pro Gly Trp  
                                     130                                    135                                    140

Phe Leu Cys Thr Ala Met Glu Ala Asp Gln Pro Val Ser Leu Thr Asn  
 145                                    150                                    155                                    160

Met Pro Asp Glu Gly Val Met Val Thr Lys Phe Tyr Phe Gln Glu Asp  
                                     165                                    170                                    175

Glu

&lt;210&gt; 10

&lt;211&gt; 155

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 10

Met Val Leu Ser Gly Ala Leu Cys Phe Arg Met Lys Asp Ser Ala Leu  
 1 5 10 15

Lys Val Leu Tyr Leu His Asn Asn Gln Leu Leu Ala Gly Gly Leu His  
 20 25 30

Ala Gly Lys Val Ile Lys Gly Glu Glu Ile Ser Val Val Pro Asn Arg  
 35 40 45

Trp Leu Asp Ala Ser Leu Ser Pro Val Ile Leu Gly Val Gln Gly Gly  
 50 55 60

Ser Gln Cys Leu Ser Cys Gly Val Gly Gln Glu Pro Thr Leu Thr Leu  
 65 70 75 80

Glu Pro Val Asn Ile Met Glu Leu Tyr Leu Gly Ala Lys Glu Ser Lys  
 85 90 95

Ser Phe Thr Phe Tyr Arg Arg Asp Met Gly Leu Thr Ser Ser Phe Glu  
 100 105 110

Ser Ala Ala Tyr Pro Gly Trp Phe Leu Cys Thr Val Pro Glu Ala Asp  
 115 120 125

Gln Pro Val Arg Leu Thr Gln Leu Pro Glu Asn Gly Gly Trp Asn Ala  
 130 135 140

Pro Ile Thr Asp Phe Tyr Phe Gln Gln Cys Asp  
 145 150 155

&lt;210&gt; 11

&lt;211&gt; 178

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

Met Ser Phe Val Gly Glu Asn Ser Gly Val Lys Met Gly Ser Glu Asp  
 1 5 10 15

Trp Glu Lys Asp Glu Pro Gln Cys Cys Leu Glu Asp Pro Ala Gly Ser  
 20 25 30

Pro Leu Glu Pro Gly Pro Ser Leu Pro Thr Met Asn Phe Val His Thr  
 35 40 45

Lys Ile Phe Phe Ala Leu Ala Ser Ser Leu Ser Ser Ala Ser Ala Glu  
 50 55 60

Lys Gly Ser Pro Ile Leu Leu Gly Val Ser Lys Gly Glu Phe Cys Leu  
 65 70 75 80

Tyr Cys Asp Lys Asp Lys Gly Gln Ser His Pro Ser Leu Gln Leu Lys  
 85 90 95

Lys Glu Lys Leu Met Lys Leu Ala Ala Gln Lys Glu Ser Ala Arg Arg  
 100 105 110

Pro Phe Ile Phe Tyr Arg Ala Gln Val Gly Ser Trp Asn Met Leu Glu  
 115 120 125

Ser Ala Ala His Pro Gly Trp Phe Ile Cys Thr Ser Cys Asn Cys Asn  
 130 135 140

Glu Pro Val Gly Val Thr Asp Lys Phe Glu Asn Arg Lys His Ile Glu  
 145 150 155 160

Phe Ser Phe Gln Pro Val Cys Lys Ala Glu Met Ser Pro Ser Glu Val  
 165 170 175

Ser Asp

<210> 12  
 <211> 218  
 <212> PRT  
 <213> Homo sapiens

<400> 12  
 Met Ser Phe Val Gly Glu Asn Ser Gly Val Lys Met Gly Ser Glu Asp  
 1 5 10 15

Trp Glu Lys Asp Glu Pro Gln Cys Cys Leu Glu Asp Pro Ala Gly Ser  
 20 25 30

Pro Leu Glu Pro Gly Pro Ser Leu Pro Thr Met Asn Phe Val His Thr  
 35 40 45

Ser Pro Lys Val Lys Asn Leu Asn Pro Lys Lys Phe Ser Ile His Asp  
 50 55 60

Gln Asp His Lys Val Leu Val Leu Asp Ser Gly Asn Leu Ile Ala Val  
 65 70 75 80

Pro Asp Lys Asn Tyr Ile Arg Pro Glu Ile Phe Phe Ala Leu Ala Ser  
 85 90 95

Ser Leu Ser Ser Ala Ser Ala Glu Lys Gly Ser Pro Ile Leu Leu Gly  
 100 105 110

Val Ser Lys Gly Glu Phe Cys Leu Tyr Cys Asp Lys Asp Lys Gly Gln  
 115 120 125

Ser His Pro Ser Leu Gln Leu Lys Lys Glu Lys Leu Met Lys Leu Ala  
 130 135 140

Ala Gln Lys Glu Ser Ala Arg Arg Pro Phe Ile Phe Tyr Arg Ala Gln  
 145 150 155 160

Val Gly Ser Trp Asn Met Leu Glu Ser Ala Ala His Pro Gly Trp Phe  
 165 170 175

Ile Cys Thr Ser Cys Asn Cys Asn Glu Pro Val Gly Val Thr Asp Lys  
 180 185 190

Phe Glu Asn Arg Lys His Ile Glu Phe Ser Phe Gln Pro Val Cys Lys  
 195 200 205



Ala Glu Met Ser Pro Ser Glu Val Ser Asp  
210 215

<210> 13  
<211> 192  
<212> PRT  
<213> Homo sapiens

<400> 13  
Met Ser Gly Cys Asp Arg Arg Glu Thr Glu Thr Lys Gly Lys Asn Ser  
1 5 10 15  
Phe Lys Lys Arg Leu Arg Gly Pro Lys Val Lys Asn Leu Asn Pro Lys  
20 25 30  
Lys Phe Ser Ile His Asp Gln Asp His Lys Val Leu Val Leu Asp Ser  
35 40 45  
Gly Asn Leu Ile Ala Val Pro Asp Lys Asn Tyr Ile Arg Pro Glu Ile  
50 55 60  
Phe Phe Ala Leu Ala Ser Ser Leu Ser Ser Ala Ser Ala Glu Lys Gly  
65 70 75 80  
Ser Pro Ile Leu Leu Gly Val Ser Lys Gly Glu Phe Cys Leu Tyr Cys  
85 90 95  
Asp Lys Asp Lys Gly Gln Ser His Pro Ser Leu Gln Leu Lys Lys Glu  
100 105 110  
Lys Leu Met Lys Leu Ala Ala Gln Lys Glu Ser Ala Arg Arg Pro Phe  
115 120 125  
Ile Phe Tyr Arg Ala Gln Val Gly Ser Trp Asn Met Leu Glu Ser Ala  
130 135 140  
Ala His Pro Gly Trp Phe Ile Cys Thr Ser Cys Asn Cys Asn Glu Pro  
145 150 155 160  
Val Gly Val Thr Asp Lys Phe Glu Asn Arg Lys His Ile Glu Phe Ser  
165 170 175  
Phe Gln Pro Val Cys Lys Ala Glu Met Ser Pro Ser Glu Val Ser Asp  
180 185 190

<210> 14  
<211> 169  
<212> PRT  
<213> Homo sapiens

<400> 14  
Met Arg Gly Thr Pro Gly Asp Ala Asp Gly Gly Gly Arg Ala Val Tyr  
1 5 10 15  
Gln Ser Met Cys Lys Pro Ile Thr Gly Thr Ile Asn Asp Leu Asn Gln

	20		25		30	
Gln Val Trp Thr Leu Gln Gly Gln Asn Leu Val Ala Val Pro Arg Ser	35		40		45	
Asp Ser Val Thr Pro Val Thr Val Ala Val Ile Thr Cys Lys Tyr Pro	50		55		60	
Glu Ala Leu Glu Gln Gly Arg Gly Asp Pro Ile Tyr Leu Gly Ile Gln	65		70		75	80
Asn Pro Glu Met Cys Leu Tyr Cys Glu Lys Val Gly Glu Gln Pro Thr		85		90		95
Leu Gln Leu Lys Glu Gln Lys Ile Met Asp Leu Tyr Gly Gln Pro Glu		100		105		110
Pro Val Lys Pro Phe Leu Phe Tyr Arg Ala Lys Thr Gly Arg Thr Ser		115		120		125
Thr Leu Glu Ser Val Ala Phe Pro Asp Trp Phe Ile Ala Ser Ser Lys		130		135		140
Arg Asp Gln Pro Ile Ile Leu Thr Ser Glu Leu Gly Lys Ser Tyr Asn		145		150		155 160
Thr Ala Phe Glu Leu Asn Ile Asn Asp		165				

&lt;210&gt; 15

&lt;211&gt; 208

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 15

Met Arg Gly Thr Pro Gly Asp Ala Asp Gly Gly Gly Arg Ala Val Tyr	1		5		10		15
Gln Ser Ser Glu Ser Asn Ala Val Gly Met Gly Leu Trp Arg Leu Arg		20		25		30	
Pro Ser Ala Leu Thr Leu Ser Pro Val Glu Ala Pro Ala Phe Ser Ala		35		40		45	
Pro Leu Cys Thr Leu Pro Phe Pro Pro Val Cys Lys Pro Ile Thr Gly		50		55		60	
Thr Ile Asn Asp Leu Asn Gln Gln Val Trp Thr Leu Gln Gly Gln Asn		65		70		75	80
Leu Val Ala Val Pro Arg Ser Asp Ser Val Thr Pro Val Thr Val Ala		85		90		95	
Val Ile Thr Cys Lys Tyr Pro Glu Ala Leu Glu Gln Gly Arg Gly Asp		100		105		110	
Pro Ile Tyr Leu Gly Ile Gln Asn Pro Glu Met Cys Leu Tyr Cys Glu		115		120		125	
Lys Val Gly Glu Gln Pro Thr Leu Gln Leu Lys Glu Gln Lys Ile Met							

130	135	140
Asp Leu Tyr Gly Gln Pro Glu Pro Val Lys Pro Phe Leu Phe Tyr Arg		
145	150	155 160
Ala Lys Thr Gly Arg Thr Ser Thr Leu Glu Ser Val Ala Phe Pro Asp		
	165	170 175
Trp Phe Ile Ala Ser Ser Lys Arg Asp Gln Pro Ile Ile Leu Thr Ser		
	180	185 190
Glu Leu Gly Lys Ser Tyr Asn Thr Ala Phe Glu Leu Asn Ile Asn Asp		
	195	200 205

<210> 16  
 <211> 158  
 <212> PRT  
 <213> Homo sapiens

<400> 16  
 Met Glu Lys Ala Leu Lys Ile Asp Thr Pro Gln Gln Gly Ser Ile Gln  
 1 5 10 15

Asp Ile Asn His Arg Val Trp Val Leu Gln Asp Gln Thr Leu Ile Ala  
 20 25 30

Val Pro Arg Lys Asp Arg Met Ser Pro Val Thr Ile Ala Leu Ile Ser  
 35 40 45

Cys Arg His Val Glu Thr Leu Glu Lys Asp Arg Gly Asn Pro Ile Tyr  
 50 55 60

Leu Gly Leu Asn Gly Leu Asn Leu Cys Leu Met Cys Ala Lys Val Gly  
 65 70 75 80

Asp Gln Pro Thr Leu Gln Leu Lys Glu Lys Asp Ile Met Asp Leu Tyr  
 85 90 95

Asn Gln Pro Glu Pro Val Lys Ser Phe Leu Phe Tyr His Ser Gln Ser  
 100 105 110

Gly Arg Asn Ser Thr Phe Glu Ser Val Ala Phe Pro Gly Trp Phe Ile  
 115 120 125

Ala Val Ser Ser Glu Gly Gly Cys Pro Leu Ile Leu Thr Gln Glu Leu  
 130 135 140

Gly Lys Ala Asn Thr Thr Asp Phe Gly Leu Thr Met Leu Phe  
 145 150 155

<210> 17  
 <211> 157  
 <212> PRT  
 <213> Homo sapiens

<400> 17

Met Asn Pro Gln Arg Glu Ala Ala Pro Lys Ser Tyr Ala Ile Arg Asp  
 1 5 10 15

Ser Arg Gln Met Val Trp Val Leu Ser Gly Asn Ser Leu Ile Ala Ala  
 20 25 30

Pro Leu Ser Arg Ser Ile Lys Pro Val Thr Leu His Leu Ile Ala Cys  
 35 40 45

Arg Asp Thr Glu Phe Ser Asp Lys Glu Lys Gly Asn Met Val Tyr Leu  
 50 55 60

Gly Ile Lys Gly Lys Asp Leu Cys Leu Phe Cys Ala Glu Ile Gln Gly  
 65 70 75 80

Lys Pro Thr Leu Gln Leu Lys Glu Lys Asn Ile Met Asp Leu Tyr Val  
 85 90 95

Glu Lys Lys Ala Gln Lys Pro Phe Leu Phe Phe His Asn Lys Glu Gly  
 100 105 110

Ser Thr Ser Val Phe Gln Ser Val Ser Tyr Pro Gly Trp Phe Ile Ala  
 115 120 125

Thr Ser Thr Thr Ser Gly Gln Pro Ile Phe Leu Thr Lys Glu Arg Gly  
 130 135 140

Ile Thr Asn Asn Thr Asn Phe Tyr Leu Asp Ser Val Glu  
 145 150 155

&lt;210&gt; 18

&lt;211&gt; 11

&lt;212&gt; PRT

&lt;213&gt; Human immunodeficiency virus type 1

&lt;400&gt; 18

Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg  
 1 5 10

&lt;210&gt; 19

&lt;211&gt; 15

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: internalizing  
 domain derived from HIV tat protein

&lt;400&gt; 19

Gly Gly Gly Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg  
 1 5 10 15

&lt;210&gt; 20

&lt;211&gt; 23

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:  
Oligonucleotide 2349-98

<400> 20  
cacacgcttc accttctttc cag 23

<210> 21  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide 2349-99

<400> 21  
taaaacttgg tacgggctga ggg 23

<210> 22  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide 1572-36

<400> 22  
gtgtggaatt gtgagcggat aac 23

<210> 23  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide 2328-91

<400> 23  
ctatgaccat gattacgcca agc 23

<210> 24  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide 2351-47

<400> 24  
gctgtactgg ctgctggggc 20

<210> 25  
<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:  
Oligonucleotide 2351-48

<400> 25

ccttcaggct tgaggctgct g

21

<210> 26

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:  
Oligonucleotide 2329-93

<400> 26

cgggectctt cgctattacg c

21

<210> 27

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:  
Oligonucleotide 2363-04

<400> 27

cctggctggt tcctgtgtgg c

21

<210> 28

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:  
Oligonucleotide 2329-94

<400> 28

tggcgaaagg gggatgtgct g

21

<210> 29

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:  
Oligonucleotide AP-1

<400> 29

ccatcctaatac gactcact atagggc

27

<210> 30  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide 2353-87

<400> 30  
ccttggtgag ctgtactggc tg 22

<210> 31  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide 2349-52

<400> 31  
ccgggccaca caggaacca 19

<210> 32  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide 2349-51

<400> 32  
aagaggccac acgcttcacc ttct 24

<210> 33  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide 2557-95

<400> 33  
aagccttttt cttcttttgcc tcaagt 26

<210> 34  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:

## Oligonucleotide 2557-96

&lt;400&gt; 34

tgccatttaa tgtaacacgg tcacag

26

&lt;210&gt; 35

&lt;211&gt; 459

&lt;212&gt; DNA

&lt;213&gt; Mus musculus

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(459)

&lt;400&gt; 35

atg	tgc	tcc	ctt	ccc	atg	gca	aga	tac	tac	ata	atc	aag	gat	gca	cat	48
Met	Cys	Ser	Leu	Pro	Met	Ala	Arg	Tyr	Tyr	Ile	Ile	Lys	Asp	Ala	His	
1				5				10					15			

caa	aag	gct	ttg	tac	aca	cgg	aat	ggc	cag	ctc	ctg	ctg	gga	gac	cct	96
Gln	Lys	Ala	Leu	Tyr	Thr	Arg	Asn	Gly	Gln	Leu	Leu	Leu	Gly	Asp	Pro	
		20						25					30			

gat	tca	gac	aat	tat	agt	cca	gag	aag	gtc	tgt	atc	ctt	cct	aac	cga	144
Asp	Ser	Asp	Asn	Tyr	Ser	Pro	Glu	Lys	Val	Cys	Ile	Leu	Pro	Asn	Arg	
		35					40					45				

ggc	cta	gac	cgc	tcc	aag	gtc	ccc	atc	ttc	ctg	ggg	atg	cag	gga	gga	192
Gly	Leu	Asp	Arg	Ser	Lys	Val	Pro	Ile	Phe	Leu	Gly	Met	Gln	Gly	Gly	
	50					55					60					

agt	tgc	tgc	ctg	gcg	tgt	gta	aag	aca	aga	gag	gga	cct	ctc	ctg	cag	240
Ser	Cys	Cys	Leu	Ala	Cys	Val	Lys	Thr	Arg	Glu	Gly	Pro	Leu	Leu	Gln	
65					70					75				80		

ctg	gag	gat	gtg	aac	atc	gag	gac	cta	tac	aag	gga	ggt	gaa	caa	acc	288
Leu	Glu	Asp	Val	Asn	Ile	Glu	Asp	Leu	Tyr	Lys	Gly	Gly	Glu	Gln	Thr	
				85					90					95		

acc	cgt	ttc	acc	ttt	ttc	cag	aga	agc	ttg	gga	tct	gcc	ttc	agg	ctt	336
Thr	Arg	Phe	Thr	Phe	Phe	Gln	Arg	Ser	Leu	Gly	Ser	Ala	Phe	Arg	Leu	
		100						105					110			

gag	gct	gct	gcc	tgc	cct	ggc	tgg	ttt	ctc	tgt	ggc	cca	gct	gag	ccc	384
Glu	Ala	Ala	Ala	Cys	Pro	Gly	Trp	Phe	Leu	Cys	Gly	Pro	Ala	Glu	Pro	
		115				120					125					

cag	cag	cca	gtg	cag	ctc	acc	aaa	gag	agt	gaa	ccc	tcc	acc	cat	act	432
Gln	Gln	Pro	Val	Gln	Leu	Thr	Lys	Glu	Ser	Glu	Pro	Ser	Thr	His	Thr	
		130				135				140						

gaa	ttc	tac	ttt	gag	atg	agt	cgg	taa								459
Glu	Phe	Tyr	Phe	Glu	Met	Ser	Arg									
145					150											

&lt;210&gt; 36

&lt;211&gt; 152

&lt;212&gt; PRT

&lt;213&gt; Mus musculus



&lt;400&gt; 36

Met Cys Ser Leu Pro Met Ala Arg Tyr Tyr Ile Ile Lys Asp Ala His  
 1 5 10 15

Gln Lys Ala Leu Tyr Thr Arg Asn Gly Gln Leu Leu Leu Gly Asp Pro  
 20 25 30

Asp Ser Asp Asn Tyr Ser Pro Glu Lys Val Cys Ile Leu Pro Asn Arg  
 35 40 45

Gly Leu Asp Arg Ser Lys Val Pro Ile Phe Leu Gly Met Gln Gly Gly  
 50 55 60

Ser Cys Cys Leu Ala Cys Val Lys Thr Arg Glu Gly Pro Leu Leu Gln  
 65 70 75 80

Leu Glu Asp Val Asn Ile Glu Asp Leu Tyr Lys Gly Gly Glu Gln Thr  
 85 90 95

Thr Arg Phe Thr Phe Phe Gln Arg Ser Leu Gly Ser Ala Phe Arg Leu  
 100 105 110

Glu Ala Ala Ala Cys Pro Gly Trp Phe Leu Cys Gly Pro Ala Glu Pro  
 115 120 125

Gln Gln Pro Val Gln Leu Thr Lys Glu Ser Glu Pro Ser Thr His Thr  
 130 135 140

Glu Phe Tyr Phe Glu Met Ser Arg  
 145 150

&lt;210&gt; 37

&lt;211&gt; 13017

&lt;212&gt; DNA

&lt;213&gt; Mus musculus

&lt;220&gt;

&lt;221&gt; exon

&lt;222&gt; (8025) .. (8054)

&lt;220&gt;

&lt;221&gt; exon

&lt;222&gt; (9548) .. (9631)

&lt;220&gt;

&lt;221&gt; exon

&lt;222&gt; (9967) .. (10092)

&lt;220&gt;

&lt;221&gt; exon

&lt;222&gt; (10358) .. (10570)

&lt;400&gt; 37

actagtctcc catagacaac agctgaatgt acgaggctcag aagcaaggcc tgccccagaa 60

ccattgcaag ccagggtgctg tcttgattgt agcctcataa aaaactgatg cagaattgcc 120

ccaccaacat gctccagatt cctgctccac agaaaccctg tgaactaacc atgttgcttt 180

tagattctgc agtaagttga taatctgcag taaataacat tcgatgaaag agaaacatgt 240  
gtagttactt tattatgac aaaactttat ttctccactc ttccattttt ccttctcaga 300  
attgacacca gccttttact aacccaaata gcctatttaa atgctgatca tactttctctt 360  
gttaactgtt acctgttccc aaaaggtaca attccctttc gaccatagct gcctctccca 420  
cctgcacacc aggatgtttc tcatattttc acctaaaaca ttggggacta caagtgaag 480  
caaaagaggg ggtccatctc agaaccccag gtatttagct gtaaaactca cttgtcaggg 540  
cagcttgaca ggtttacagt ttgtagaagg accagaaaga aggtagccaa gacagaagag 600  
gcaacctctg cttgtcctag aaccttcagt ccatatacat ctaagctccc cagcaccatt 660  
tctaccacag acctctcaga gttcctgagg atgcagaccc caggacactg acctcagttt 720  
ccaggcaggg ttctctgaca ccccttcac actgcctgac tgggagttag tctcatgggtg 780  
caacactact ttgggacact gtacccatcc cctcgaccta cagaaacctt tcaacttttca 840  
aggtcacctc ctataggaag tatttgaaaa gatgagagtc atgggtcattt gctatgataa 900  
tattctgtgc ttatctccct gtaaaaagtt ggcttggggg ctctggcatg catctgacct 960  
taaggttgga gctgcaccaa tatgttttta agcaccggc ataatgcttc gcaaaatttc 1020  
agaacatggg ttgtacagaa tgtactttcc tccactcata caaaccttg taaaagagta 1080  
gtttgaatcc caactcattc ttgaaggcca cttttttag ggtgacagaa tttaaaaata 1140  
cagaatttaa aaatacttta tcccaggga gctcacactt ctaaaccag aatgaaagaa 1200  
gaaatagaaa cacacttggt gtggcggtgg tgggtggtgat ggtggtcgtg gtgggtggtg 1260  
tgggtggtggt ggtgatggtg gtgggtggtg tgggtggtggt ggtcgtggtg gtgtaatgat 1320  
cacagtaaag tgaggcatca tggcctgaga gagtcaggca tcacagctat tcaagtgaag 1380  
actacctact actgatttta gagttctata attttagtag cagccacagg cctggggcct 1440  
gggcctatat ttccagagag gaaatgttca cagcaggtca actgcagaca gtgaagatca 1500  
gaaatgtttc ataatcaggt catcagagaa aaggcaaagg agctgatgga ctttatcctg 1560  
aaaaagcaaa atccaacca cctcatgctt aatgcattca aaggtctgcg ggcagaagaa 1620  
tacattttgc tttttattat tataaattac ctggagaata tttttgtctg aattatctcc 1680  
caaataattaa ccataaaaat aaaaaattcc atgtgtgctt ctcccagggg ctataaagcc 1740  
cctgggtctta gagttgttgg ggcaaacct gacctttgaa gtagttactt ttgaagatgc 1800  
cataccatac atttggccac ttggagagag tctaagtca catctaaagg gttactctga 1860  
tgctctgttt tctcatatgc ccttggttta cagctaacta tggctccagc taaactataa 1920  
agttccttgg caacagagat ggtacgctat gtgtctttga cacagcagaa taaatgctta 1980  
gtgaacatta ctgattgcct gacaggacac ctcacacttt ggtactttca acagagggat 2040

gtaaacttat gaagaacaat gaagaatgaa tattggcaat aaaagcaaaa attggttaac 2100  
ccaattctag ctctgaaatc attttttaggt agtgggaagt ctttttgttt tgtttattca 2160  
ctttacatcc caattgctgt cctccctcca agttccccac caccaccaca gtcccttttc 2220  
cctccccctc tectctgaga gaatggagaa cctcctgga tattccccca tcatgaaaca 2280  
ttaagtctct gcagggctag acacttcccc cagtgaggcc agtcaggga gccagctag 2340  
aaaaagcata tcccacagac agacaacagc ttttgggata gccccgttcc agttgttttag 2400  
gatccacatg aaggctgagc tgcacatctg ctacatatga atgaggaggc ctaggtccag 2460  
cctgtgtatg ttctttgggt ggtggttcag actctgagag cccaagggt ccaggctcagt 2520  
tgactctgtt ggtcttctg tggacacct gtccccctcc agcccacaat ccttccccta 2580  
atccttctcc ttctacttc cataagagtg tgaggagtct ttaaaaacat gaagcatttt 2640  
atctccccag ggcaacacat ggaaatgaaa gattgtgaaa agtaatttaa agaaaaagaa 2700  
aaaaaaattt aacaaggaat aagaatcttg tttctctgaa aatgcttaag agtgtggaaa 2760  
acataaactg gattctaata gaatgcaatt ggattgtaat gaaaacctat caaagttatg 2820  
aaatagcttt cactacctg cacaaaatct ctggcatgt gtgtgtgttg caaattttct 2880  
tgtagttta aaaccacaac aataacaaca aaatagcaaa aattgggtct cagcctcatt 2940  
cattttttct catttcttg cctgtgatcg tctgggtctt aagctgacac ctcaccaatt 3000  
cctcatcaag acctttgtg aaatttgcaa atgtcccaaa aaggagaatt acaataagtc 3060  
agagaacgtt ctgtccaatt ctttatccct agtgatggat gagtaaagga tgtataagag 3120  
atggataaat ggactgatgt acagataaat gaaggaatat gtacatgggt aggtggatag 3180  
atgacttact caacagatga gtagaaggat gagaaataga tggacagctg gactgaggca 3240  
tgcaaagtca actggagaac tgagtctctt gaccatgcac tgtccagggt ctcattatcc 3300  
ctagagtcca gggcccatgg ctctgtgcc atccccatgc aaatctaagg ttaatacgtt 3360  
ctacagctga gtttcttac atatgtgtct cagtaagttt gtatcaacta attaaatctg 3420  
aaaggagttc cttctgatct tcccaaacag agccacactc gtgatgaagt cagccctgct 3480  
tcattgtggt tctctggatg catctggctt ccatcagcat aatctttcta ttcttgatcc 3540  
ttccaacctc ttcaggctc agacagaacc ccatggagca tcaaagaggt ttgacccag 3600  
cattgtttat gtagctgcaa aaccactaat aacacagtca atgacagtag ctacagagac 3660  
agcaggctcag tgtctggcct ctgtcaaggc tttatgagt actctctccc ctccccgcaa 3720  
atactcatta atctccccac ctcttatta tttggactgt gttgaagata ttatgaaatc 3780  
tctgggtctt tcttcccgga tctagagcca attacagatt ctgtaggttt gaccaccct 3840

gaccagacat tataaacaca gtgctggtgc cctgaagaaa acagttggag actccaggca 3900  
ttagaatcca ggcaccagga actacaggtc agtggtgaca gtcggtctct ctgtgtatct 3960  
cttacacaca cacacatata cacacacaac acaacatata cacacatata acacacaaca 4020  
catacacata caacacatac acacacacaa cacttttctg taatgtctcc aaaattctca 4080  
ggctctaggg aagaagaaat gtctttttaga gaatgcggtg tgatgttcta taagtctagg 4140  
aatacttgat agaatttaat gagaagtata gattagggtca aagcaagggt actacatatt 4200  
tggaaccaca gagttttgaa agtcattctca aaagaaatta tttaggccag agatgttcaa 4260  
aaaatgtttt gtttgtgaca tatggaagct cccatggaga cattctgtga ttctcatcaa 4320  
tagacagtag ggatgccacc aagggtgctaa cgtcttcctc accccatcat ctatcataca 4380  
tccaaatggg ttctttgaaa acaatctcct tgtgaaactt aaagtagcct tgaaaatata 4440  
ataatcttgt ccagcctctc atttcaatgg gaatagattg aaggcctaag gaccaaaca 4500  
aaaaacaaaa caaacaaat aaaaacacca aaaaaaaac ccataaaatg aatgagtagc 4560  
taagttattt ttagaatcca gcctttcagt caaagcttga ttcattgata tctgtgttct 4620  
gatcttaagg tgctgtgtct gtcagttgta tagttggata gaggtacaga tgagctatat 4680  
acatcatgct tcaagatttc aggatcttat aacttttata aagcaaataa tttgtcttaa 4740  
tgcacactaa taaacaatat agcaaagttt gacaggagtt cagagtactg ttagagaagt 4800  
gaagggaaga attttggtat gatagtaaag gggaaaatca aattttgagt catggaatca 4860  
tacatagttt gacatagaaa gaaccttggc aaccacataa tctaattgat gagcccaaga 4920  
actggcctgt gtttttaaga tctcattctc agctgttatg taactgaaca gacaagatac 4980  
taagcccaag tatagtgaag ccattgtccag tgatcttaat aggagtgaac ggaatgggtg 5040  
gtgatgaaga ggggtggatt ttgagcagga ataccaaaag caatgctgac tgtgcccttg 5100  
gagagaatta gcatgagtc ttgagagaaa aatgagatgc tattgcacaa gcaacctagg 5160  
gccagatggg gtcaagatag gtggccatcg tggactttag aaccaggcag gaatgtgatc 5220  
agagatgtac tttatgtagg ttaggtttga ttcagaaacc aggagggtta gcatgtttac 5280  
aatggtgact aaaaacaagc acaaggttat actttaaaaga aataatctct gaaaagaagg 5340  
gaggtatatt ttcagtgccg gaaagaggaa tattacaaaa gtgagaggag tagatttgag 5400  
aaagagaagt ggattgtgga ggagcagatg ctcaccacgc ccttacactc acttgaactg 5460  
acacccaaag atgaagggtg gctgtggact gctgaagctc agcctgtggc tgggaagcag 5520  
taaacaaaat tgctcatcac agctgtacaa gatattccat agcatataaa aataaaagtg 5580  
cttaggctat tctcttaca ctctcagcct tatgaatgac ccggaaggaa aagaactcta 5640  
caatgtgcct gtgtctgttc ttacttcctc tgccacaagc aaaagagcct tgggaattgg 5700

ctcagagggg acgtcatcaa acaggctggc cttgaggctg ggctgttatt cgtctacctg 5760  
ggatagagga attcgctatt cttttataat ccaagtgtgg cctggggacc agcagcatta 5820  
ttaagacctg gttgcatgtt tgaaatgcag tctcagattt catcccagac ctaaagagta 5880  
acactgtttt catgaggata caagattaag aaatatgcat tagagagtaa ttggctaaat 5940  
gggtaaatgt catgcaagca ggaggatctg attgactccc caggaccac acagttccca 6000  
tgccgtagag cacatctgta atcacagtag gcgtatgatg aaatgggagg tgaatcaaga 6060  
gaatctctag cagctacggg ctggccagcc tcccatgcac agcactaaat aaggcaagga 6120  
ccaataacctg aagttgtccc attaccttca catatacacc acggcatgtg tgtacttgta 6180  
ctcacacata caaacaata cacacgtgca cacatacaaa actcagagat taaggacaat 6240  
tggcctgaca tatcagttcc taagcctggc tcattgcttg taacactaca agcagtatta 6300  
aataaggata ggcgagagaa cagttaccga atggttcaga agtggggcca tgctgtgac 6360  
tttaaacaaa tgtttcatat ttttaaataa taacacttag attacaaaat aaatttacta 6420  
caggaaaatg ttaagaacta tcaacaacca ttgactatcc tgtcggccac aaatgagtgt 6480  
tataacaagc accagccgtc cttgtccaca tgtgtgtgtg tctacacagc tatgaattta 6540  
attgggataa taatgtgcac attctttacg gcctgcagtt tttacttcat gtatttgaaa 6600  
tgtttgtgcc acaaagtca tctttaagga gcatatcctt atttcctgga tttatcattc 6660  
cctttcagcc gactggacat tgacagcatt tccaactttt caaccttgta aaaataacta 6720  
attgaactat tttataacta agcatttggg caatcaatta cctctgcctg gaatgggggc 6780  
aacaacacat gcaatcatgg gaaagccagg atgctgctgt ctgatcccta gccctggcat 6840  
tcgtgcagaa cctcactctc atctgtgcc tgatatcctt cactctcaag tcttttccca 6900  
gtgactttta aaggcaacag aatcatatag ccaataatga aagctacttg gtctacagtt 6960  
gtgtggcggt ttttatagat attttcttca tttacatttc aaatgctatc ccaaaagtcc 7020  
cctataccct cccccacct gctcccctac ccactcactc ccacttcttg gccctggctt 7080  
tcccccttac tggggcatat aaagtttgct agaccaaggg gcctctcttc ccaatgatgg 7140  
ccaactaggc cattttctgc tacatatgca gctagagaca ccagttctgg ggttactggt 7200  
tagttcatat tgttgttcta cctatggggg tgcagacccc ttcagctctt gagtactttc 7260  
tctagctcct ccattgggag ccctgtgttc catcctatag atgactgtga gcatccactt 7320  
ctgtatttgc caggcactgg catatgaaat agtatctgca tttggtggct gattatggga 7380  
tggaaccccg ggtggggcag tctctggatg gtccatcctt tcatcttagc tccaaacttt 7440  
gtctctgcaa cttcttccat ggatatttta gtccctaata tagggagaaa tgaagtatcc 7500

acaagttgat cttccttctt gattttctta tgttttagaa gttgtatctt ggatattcta 7560  
ggtttctggg ctaatatcca cttatcagtg agtacatato aagtgaattc ttttgtgatt 7620  
aggttacctc actcaagatg atattctcca ctatgttcat agcagcccta tttatagtag 7680  
ccagaagctg gaaagaaccc agtcctcaa cagaggaatg gatacagaaa atgtggcaca 7740  
tttatgcaat ggagtaccac tcagatatta aaaacaacga atttatgaaa ttctcgggca 7800  
aaacctatc taaagaccag gaataaggaa aagatggact gcctgcctgc agctgggaga 7860  
gctggggaga cttttgtgga ttctgtaata cttaggggta cggaacagct tgtggctgga 7920  
taattctgag ctccagcatg tctgcccccc aaaaaacatt ctgtttttct gaaagccttt 7980  
ttcttctttg cctcagtga gaccagacac tcccaactgc agga atg tgc tcc ctt 8036  
ccc atg gca aga tac tac atgtaagtaa tcttaacgat cgctcaatca 8084  
aggggcctgg agatcacatg agaagggaaa aggctgagtc aaagggacaa agctccctct 8144  
agccacagaa atctcaaaca ctgaataatt gatcttcato tttgtcaatc acaacagccc 8204  
tctttcctgg tgacagaatg gaacaactgt aagagtggta ttgcttagtc cattttacag 8264  
acccggaaac tcaacctcca cgaggttata caattttcct catgtcatgc aattacccaa 8324  
aagcagagag tgggatcgga ctctctgttc tctaaactga tgtagctagt tcttagaaag 8384  
ctcaaacat cttgagtccc aaggacagca ctttatggg cacctggatt gataacctata 8444  
tcaaaaaaaaa aaaaaggctc cactagatag cctgggtac cctgaaactc tcaactgtgta 8504  
catttaggtg accacgaact cacagagatc tgccttccaa gtgctgggat taaagtatgt 8564  
accaccacac ctgcatcttt gacaataact gagtgggtatc taaattcttc cagtggctaa 8624  
acagttaagt ccagttccc aaagtctgag aaaaatgccg ggtggtgaaa tctgtacaga 8684  
cctttgttct taatgtacaa gtgagcctgc tttaaaaaca atacgcaagc tgtttttgct 8744  
attgctaagt gttgcagaga cagaaaaggc tcccagaagt ggtaactttg gtccagaggt 8804  
tctgttctca aactcattgt gagctctgaa agcaactgat gggcagctct gaaatcagct 8864  
gggcaattag gctaataaca ggcataattt taatgtttca cacgcatgac agttcctccc 8924  
cagctgcctc agtacatact taccctccta ggcacgtcat tagaccata ggtataacca 8984  
gtgactaatc aggccctggg ctaattctaa gttggcctcc tatataagtg ccactcagag 9044  
tgtacctcat catggctgta gtgggcccag agtctagggg catagacttt tctattgtcc 9104  
aatttctgat ttgtgaattt tctacaaaaa gaattttttt taattttaca aatcaaatca 9164  
cagttactac atcttcagtt ccttcattaa ttagtggtac tatttaaaaa aataaaataa 9224  
atcaagctca gaaacatcat ggataggggt cattgtatct ccaggggtacc tgagcttcaa 9284  
agcaactcct cagacagcca tgaaaacatc ctcaattacc tcatgagaag acactattgt 9344

cattttctgga gcctctgata atcctgagcc taggcagctt tgggatgaaa caatttctac 9404  
ccttattgga acagtgtccc tctcctgtct ggaaacaatt caccaaaggc tccatgtggt 9464  
tgtccagtaa ggtggtatgg ggacagaaat ggacaatgat ccctgagggc agtgatccat 9524  
taaccttgcc ctctatttc aga atc aag gat gca cat caa aag gct ttg tac 9577  
aca cgg aat ggc cag ctg ctg ctg gga gac cct gat tca gac aat tat 9625  
agt cca ggtgatcttc cgggtggtggg ggtgggggag tggaggggag ggtgtggggg 9681  
gggctctctt ccagaagttg cttagtgtcc atctgccaca aggccttgat tctttccttc 9741  
aattgtgtct ctagagacat gagaatattg tcacagtgat aaggagaaga ggtaggggca 9801  
gtttcttctt gtaaaaaatg aattccattt accctgcagt ctccatacag aaacaggcca 9861  
gaggggggca gaccagtaa cttctagctg agccctacct tgcttaaaac ctgccatctg 9921  
tggteccctc actgtctgaa ttgcattctg tcttacctcc cagag aag gtc tgt atc 9978  
ctt cct aac cga ggc cta gac cgc tcc aag gtc ccc atc ttc ctg ggg 10026  
atg cag gga gga agt tgc tgc ctg gcg tgt gta aag aca aga gag gga 10074  
cct ctg ctg cag ctg gag gtgagacacc cctcctcatt gcagtcagta 10122  
ctgccactgg aacatagtga catctttgaa ccacatgtc cctctcttg tttcccatct 10182  
atctctcttt gcctccagct gagggactct agcctttggg gatgtacaga aagaacatgg 10242  
cttcggaaaa ctcttccta ttgagtcctt ctttgccaa gcctctgagg cactaagggc 10302  
tgacgtccca accaaacact catttcatct cacagctgtc tccctttccc cacag gat 10360  
gtg aac atc gag gac cta tac aag gga ggt gaa caa acc acc cgt ttc 10408  
acc ttt ttc cag aga agc ttg gga tct gcc ttc agg ctt gag gct gct 10456  
gcc tgc cct ggc tgg ttt ctg tgt ggc cca gct gag ccc cag cag cca 10504  
gtg cag ctg acc aaa gag agt gaa ccc tcc acc cat act gaa ttc tac 10552  
ttt gag atg agt cgg taa agagacataa ggctggggcc tcgtctagtg 10600  
ccccagctc gagatcttct tgctcagcat ctctggaaag cagaataagg aagataccaa 10660  
agatgtttgg gtcttaatcc ccagaatctg tgaccgtgtt acattaaatg gcaaagggat 10720  
tttttttttc cttcatggtc catttgggccc cattggaatc atctgaggcc tcatgaggag 10780  
aaggaagagg tcagagggag actggggcaa actttggtac taaaagtaac aatggagaca 10840  
gggaccataa gctgatgggt aacagtgggt tctagaaacc ggaaatgatg agagctctcc 10900  
tgacacaggt tctggatttt tctggactga agaattgtga aataatacag ctccattatt 10960  
ttaagccact gagtttgaga tcattcaatg aagctgtcat aataaaacct gtgcttcaca 11020

tacaattcaa tattggtagg caccccgggtg atttcttgga aagacatcta gggattctcc 11080  
tggatgctga ttccagggtc cagtggagtc cctgggttga agagatttca caaccagaa 11140  
catcagggtc gactcttcta aaagtccgtc gttgcacccc ttgcctgaga gcattagcaa 11200  
tttctatttc ataggaaatc tgtgtccctg cccctgctaa agcagggagc ctggaccgtc 11260  
ctgatttagt gaggggtgag ctgctggcac ttttttgtgt caccagtgtc ttaagcagt 11320  
atggagcaca aaagatcttt actgagaaga tggccatgaa gctctggcta gacaccaaga 11380  
atatgatata agcagagcta cagcacaaga tgagccaatg aggaaagcca ttcagggagg 11440  
ctaagcccag ctteccaaag ggacagctaa ccttggtc aaatgaatag gggttttcct 11500  
ggcagagaac ataggtcaag cattctaggt agaatcagca attcagaaag gtgtgagaga 11560  
ggcatggaga gctccaggca tgtctgggt atggtgtgtc attcttgttg caagaatcca 11620  
acgtctgtgg ttaaggagtt gctgaaaatt aaaataggaa aatgggtaga gtctaattgt 11680  
gaatgacttg caaaggagtt tagcccataa gtggggagct cagaggagtc atctaaggat 11740  
tgcaagcagg ggccctgtga tcattgctgg accagcctag gtgctacaga gcctaccttc 11800  
agctctgcat cctcactcac atccaggta cttcagaggt caatttctgt gctctggttc 11860  
tatgggtagc ctgaccctgt ttcattcttct tgtataactt aggcacataa gcttagggac 11920  
tggtagagtt tacttgagt attggtgaat caggcagcac caaactacaa gttgttcagg 11980  
gctttaccaa gggggcactg attggagaat tggaatgagg gtggttagaa tgcattcaga 12040  
aaacaagggg aagaaaaatt tgattgctta aagtggaaag tcccaactta aatgttagtc 12100  
agtagtttct aattacttga gtctctaatt agaggtagt tggcagtttc tggtagtta 12160  
atctaagttt cattttctta ggctatgacc attctctgag tcgcatgtta gcaatgcagt 12220  
aagaactcaa gaccagaat agcctctgtt aattatttta gcaatgatca ctcatttctg 12280  
ttgcctcta ttgagatctg ttcccatgga ccaccaggc acatcaggcc tccagtacc 12340  
aacataataa tgattgctgc acagacaaaa tatttttttt cagtatctgg tatttgctac 12400  
atttccatta gtgctggagg gaaggctaca acgaccatga aggcattggc cctgccttct 12460  
aaggacttac aatgtaatag gagccctgac attataaagt gggtcacctt gtttcaaact 12520  
gagccaaact gaggtgagg gcttagatta gtggtaggtc actttccaga catgttcagt 12580  
gctaagaaaa acacattctg gggtttagtta gatgttttag ttcatttgat aagaagcca 12640  
atgattggac tttcaacttc tggaacccat gtggtggaag agagaaccaa cttctgacca 12700  
tttgggtcat ggcacatccc ctaccatcac aagaactcac caaataaat tagaaaaatc 12760  
aagaaaaact catatcctat agacctctgg tagaattagc agaacgtgc tgtggcactt 12820  
gggatttgaa actcaaaaat ggaagaagct acttgtgacc gttcaagact ccagggaggc 12880



tcctctgaca catcccacga ctcaggctta aattccttct tctccctaga aggccacgcc 12940  
atctttctcaa ccagggcaca gatgctataa ttatgtaaat gtgtgggaga ggcacacttt 13000  
agatcttata cactagt 13017